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STARCH FORMATION IN TOBACCO PLANTS DEFICIENT IN POTASSIUM[1]

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Potassium is important in the growing of fine tobacco. Not only is it the nutrient removed from the soil in the greatest quantity, but it is essential for the vigorous growth of the plant (3). Potassium is also more important than any other element in producing good combustion of the dried leaf (1) and is necessary for choice aroma (5).

This value of potassium to the finished tobacco product has a curiously paradoxical aspect, for it has long been assumed that much of the importance of this element to any plant lay in its relation to carbohydrate synthesis or translocation. But the type of tobacco grown in the Connecticut Valley is low in carbohydrates, especially starch. Consequently, there is a special interest in the problem of the relationship of potassium to the formation of starch in tobacco plants.

It was the aim in this study, as in much recent work, to test the leaves soon after the effects of potassium deficiency had been definitely established but well before the plant had died from lack of this element. From preliminary trials with tobacco, it appeared that seedlings started in soil, transplanted to sand, and treated with a nutrient solution, could be grown normally with the usual elongation of the internodes; or that similar plants, treated in a like manner except for the absence of potassium, would show evidence of a lack of this element during the latter part of the vegetative period.

Methods

Tobacco (*Nicotiana tabacum*, var. Rosenberg)[2] was germinated and grown in quantity in sandy soil until the seedlings were about two inches tall with four small leaves, from 9 to 15 weeks depending upon the season.

[1] Contributions from the Department of Botany, Smith College, New Series, no. 5.

[2] These seeds were kindly supplied by the Connecticut Agricultural Experiment Station where the starch analyses were completed in the Department of Biochemistry. The writer is indebted to Dr. H. B. VICKERY and Dr. G. W. PUCHER of that department for their continued interest and advice.

Uniform seedlings were washed thoroughly to remove all soil, and single specimens were placed in pure quartz sand in glazed jars, each with a lateral drainage hole covered on the inside with absorbent cotton to prevent loss of sand. The complete nutrient solution was one that PUCHER[2] had found suitable for growing this strain of tobacco under similar conditions (table I). In the solution lacking potassium, the potassium phosphate was

TABLE I

COMPOSITION OF CULTURE SOLUTIONS, GRAMS PER LITER

SALTS	COMPLETE NUTRIENT SOLUTION	SOLUTION MINUS POTASSIUM
KH_2PO_4	0.442	0.0
$Ca(NO_3)_2 \cdot 4H_2O$	1.015	1.015
$MgSO_4$	0.126	0.126
$NaH_2PO_4 \cdot H_2O$	0.0	0.497
$Fe_2(C_4H_4O_6)_3$	Trace*	Trace*
H_3BO_3	Trace*	Trace*
$MnSO_4 \cdot 4H_2O$	Trace*	Trace*

* 0.5 p.p.m. of Fe, B, and Mn.

replaced by sodium phosphate. The continuous flow method of SHIVE and ROBBINS (**19**) was used, the drip being regulated to furnish about two liters of nutrient solution in twenty-four hours; this was sufficient until the last week of growth when the rate was increased. Four series of plants transplanted into sand cultures respectively in November (T_1), January (T_2), March (T_3), and May (T_4) were employed.

The plants were harvested toward the end of the vegetative period, 35–39 days after transplanting, when the control plants were about 35 inches tall. The samples were taken at 3:00 P.M. on a sunny day in January (T_1), March (T_2), April (T_3), and June (T_4). All leaves below the third node, counting down from the bud, were cut separately; these individual leaves were at once weighed and dried at 70°–80° C. and were weighed again. The dried leaves from corresponding nodes of several plants were grouped together and ground to a fine powder. Weighed samples of about 200 mg. were analyzed for starch according to the iodine precipitation method of PUCHER and VICKERY[2] (**15**) as slightly modified in previous similar work on peas. (**2**).

Results

The control plants grew normally while those starved of potassium showed the characteristic appearance resulting from a lack of this element (**5, 12, 13**). The former were tall with about twenty-five nodes per plant, while the latter were short with about sixteen nodes; consequently, a leaf at the twelfth node in one of these plants may be a basal one, or very near the bottom, while a leaf so numbered on a control plant may be near the middle.

The fresh weight per single leaf reached a maximum for all series and for both treatments in the control plants harvested in June (table II). In the plants with the complete nutrient solution, the fresh weight per leaf at corresponding nodes increased progressively through the season, whereas the plants starved of potassium varied little in this respect, and the leaves harvested in June were not as heavy as earlier ones. In most cases, for both treatments, there was an increase in weight from the younger, upper leaves down to the sixteenth or eighteenth node and then a decrease. The fresh weight per leaf was generally greater in the control plants than it was in the deficient plants; it was about twice as great for those harvested in January, and three times as much in March, April, and June.

The values for dry weight per one leaf are about one-tenth of those for the fresh weight (table II). The dry weight as percentage of fresh weight tends to decrease from the top to the base for all plants, and is generally greater in the control plants with the exception of the series harvested in March.

The percentage of starch is greater in all leaves of the control plants of the June series and in all except the topmost leaves of the April series (table II). In the March series, however, nearly all the leaves from the potassium deficient plants contained a higher concentration of starch than their corresponding controls, the values in a few cases being unusually high for this type of tobacco. The general trend for both treatments seems to be an increase in percentage of starch from the younger to the older leaves and then with a few exceptions a steady decrease from about the eighth leaf. In the control plants the proportion of starch was greater the later in the season the plants were grown. The reverse was true for those without potassium.

The amount of starch in one leaf was approximately the same in the top leaves of all plants, generally being 1.0 mg. or less at the fourth or fifth node (table II). In the leaves of the control plants, it reached its maximum at or near the sixteenth node and then decreased to the base. In the plants deprived of potassium, this maximum was at about the eighth leaf. The amount of starch per leaf was generally more in the control plants than in those starved of potassium except for the upper leaves of those harvested in March. The maximum amount of starch in a leaf of the control plants was twice that of the corresponding leaf of the experimental plants for the series harvested in March, eight times as much for the April series, and eleven times as much for the June series.

Discussion

Potassium is obviously concerned in the growth of the tobacco plant since the control plants grew normally while those without potassium were

TABLE II

FRESH WEIGHT, DRY WEIGHT, AND STARCH IN TOBACCO LEAVES

SERIES	T_2—MARCH				T_3—APRIL				T_4—JUNE			
NODE FROM TOP	FRESH WEIGHT PER ONE LEAF	DRY WEIGHT PER ONE LEAF	STARCH PERCENTAGE OF DRY WEIGHT	STARCH PER ONE LEAF	FRESH WEIGHT PER ONE LEAF	DRY WEIGHT PER ONE LEAF	STARCH PERCENTAGE OF DRY WEIGHT	STARCH PER ONE LEAF	FRESH WEIGHT PER ONE LEAF	DRY WEIGHT PER ONE LEAF	STARCH PERCENTAGE OF DRY WEIGHT	STARCH PER ONE LEAF
A COMPLETE NUTRIENT SOLUTION												
	gm.	*gm.*	%	*mg.*	*gm.*	*gm.*	%	*mg.*	*gm.*	*gm.*	%	*mg.*
4	0.22	0.03	2.09	0.63	0.23	0.03	2.39	0.65	0.19	0.03	....[a]	....[a]
5	0.35	0.05	3.24	1.62	0.47	0.08	1.26	0.97	0.29	0.05	....[a]	....[a]
6	.....	.....	.....	.....	.....	.....	.....	.....	0.55	0.01	1.95	1.87
7	0.90	0.13	2.23	2.90	1.20	0.19	2.70	5.21	1.06	0.18	1.95	3.43
8	1.59	0.20	2.84	5.68	1.95	0.28	2.84	8.04	1.61	0.24	5.80	14.09
10	.....	.....	.....	.....	.....	.....	.....	.....	3.79	0.54	4.11	22.03
11	2.82	0.30	2.62	7.86	4.81	0.65	3.62	23.39	.....	.....	.....	.....
12	.....	.....	.....	.....	.....	.....	.....	.....	6.36	0.80	4.50	36.00
15	6.13	0.59	2.37	13.98	.....	.....	.....	.....	.....	.....	.....	.....
16	.....	.....	.....	.....	8.70	0.89	3.63	32.16	.....	.....	.....	.....
17	.....	.....	.....	.....	.....	.....	.....	.....	10.73	1.11	3.50	38.85
19	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
21	.....	.....	.....	.....	9.10	0.78	2.75	21.45	.....	.....	.....	.....
22	.....	.....	.....	.....	7.77	0.63	2.47	15.56	5.73	0.49	1.07	5.28
B SOLUTION MINUS POTASSIUM												
4	0.17	0.04	2.83	1.00	0.16	0.02	3.23	0.68	0.15	0.02	....[a]	....[a]
5	0.34	0.06	4.43	2.44	0.36	0.05	1.70	0.87	0.29	0.03	....[a]	....[a]
6	0.54	0.09	5.97	5.07	.....	.....	.....	.....	0.56	0.06	1.21	0.78
7	0.76	0.12	3.28	4.03	1.03	0.13	2.81	3.60	0.80	0.08	0.68	0.56
8	0.88	0.13	5.46	7.26	1.23	0.15	2.16	3.13	1.09	0.12	2.92	3.50
10	.....	.....	.....	.....	.....	.....	.....	.....	1.37	0.14	0.82	1.16
11	1.35	0.18	3.56	6.51	2.21	0.24	1.42	3.44	.....	.....	.....	.....
12	.....	.....	.....	.....	.....	.....	.....	.....	1.20	0.12	0.25	0.29
15	2.17	0.23	1.66	3.85	.....	.....	.....	.....	.....	.....	.....	.....
16	.....	.....	.....	.....	3.49	0.35	1.17	4.12	.....	.....	.....	.....
19	.....	.....	.....	.....	2.38	0.23	0.29	0.65	.....	.....	.....	.....

[a] Too little starch to test.

stunted and exhibited other deficiency symptoms. Furthermore, the control plants displayed a seasonal response by an increase in weight from January to June while the rate of development of the plants lacking this element was nearly independent of the time of year.

The control plants had more leaves which were both larger and heavier than did those deprived of potassium. This result agrees with the writer's previous work on peas and with most of the earlier reports (**2**). WARNE (**22**) also found that addition of potassium was associated with greater number and size of leaves in the beet. He suggests that increase of this element is accompanied by greater water content of the leaf, by more cell extension, by larger cell size, and by larger leaves. GREGORY and his coworkers have found increased water content in leaves with less potassium (**7, 8, 9**). STANESCU (**20**) associates high water content with synthesis of carbohydrates, a point that will be considered later. Although both the fresh and dry weights of these tobacco leaves were generally greater when potassium was furnished, the relationship of dry weight to fresh weight is the important point that must be considered. The evidence here was not conclusive, but the general trend was toward a greater percentage of dry matter and less water in the control plants. Also, in all leaves the proportion of dry matter was greater at the top of the plant than in lower leaves, that is, there was relatively less water in the upper, younger parts where available potassium generally accumulates. With peas, the dry weight per leaf also increased toward the top of the plant. However, as PHILLIPS, SMITH, and HEPLER (**14**) state, "It is true that decreased water content is found frequently associated with potassium deficiency, but it is also possible to find plants showing definite symptoms of potassium deficiency in which no significant differences in water content occur." Consequently, agreement is with them, rather than with SCHMALFUSS (**18**), in their conclusion that "the effect of potassium on the water relations of the plant is very important; but that this is its only function [as indicated by SCHMALFUSS] seems hardly compatible with the highly specific nature of the potassium requirement." ECKSTEIN (**4**) also has called attention to several critical aspects of this problem but concluded that there is still much to be done on the effect of potassium on the water economy of plants.

An indication for the need of potassium in the formation of carbohydrates is seen in the fact that, in each series, the maximal amount of starch in leaves of the control plants was greater than the maximum in the leaves of those deprived of potassium. This evidence, however, is not as conclusive as seemed to be the case in the work with peas (**2**). The amount of starch was generally more in the control tobacco plants than in those without potassium in most of the two later series, but there was a certain discrepancy in some leaves of the earlier series. RUSSELL (**17**) finds that

deficiency of potassium greatly reduces the rate of assimilation of carbon dioxide in barley. Similarly, WHITE (**23**) concludes that potassium starvation results in a low rate of assimilation in Lemna, but he also finds that it induces high starch content and high dry weight per unit area. As in the writer's work on peas, the lower tobacco leaves, but not the lowest, showed the greatest differences between the amount of starch in the control plants and that in the potassium deficient ones. When there is such a deprivation in barley, according to GREGORY and BAPTISTE (**7**), the sugar level consistently falls with the age of the leaf. All the uppermost tobacco leaves were more or less similar in storage of starch, and potassium is generally considered to be mobile, going toward the top, so it is possible that any available potassium may have moved upward and created similar conditions there in all the very young leaves with similar results as evidenced by like size, dry weight, and storage of starch.

With more daylight later in the season, the greater amount of starch in the leaves of the control plants is not unexpected, but this observation may raise the issue as to whether the carbohydrate activity is a cause or a result of better vegetative growth at this time. Since the reverse is true in the leaves of the plants starved of potassium, the question is raised whether or not these plants may be using carbohydrates for growth rather than merely for starch storage.

Curves plotted for each series to show the dry weight per leaf and the starch per leaf for all the plants grown at the same time with the same treatment are all closely alike for the control plants, although there is a slightly greater increase in starch than in dry weight as the season progresses. In the plants starved of potassium, the growth curves are less alike, as are the starch curves, and the two sets are distinctly unlike each other.

The greatest dry weight is not always observed in the leaf which is richest in starch. The leaf which shows the maximal percentage of starch is usually situated a few nodes higher than the leaf that shows maximal dry weight. It appears, therefore, that there are a few nodes at which, although the leaves are not yet fully grown, maximal starch storage is taking place. The most active photosynthesis thus occurs in these younger leaves. These nodes are a little farther apart in the plants deprived of potassium. Does this mean that the carbohydrate is being used for growth, with less storage, in the control plants? And is the carbohydrate stored rather than being used for growth in the potassium deficient plants?

Undoubtedly, these questions are closely related to the normal growth of the plant, and recent evidence indicates that a proper balance between potassium and nitrogen is important in this connection. GASSNER and GOEZE (**6**) state that assimilation is poorer with lack of balance of these

two elements than it is with the same degree of deficiency of both elements. HOFFER (**10**) and PHILLIPS, SMITH, and HEPLER (**14**) have reviewed the pertinent literature on this phase of the problem. The latter authors found that low potassium supply while there is a high level of nitrogen depresses growth and reduces the amount of starch in stems, but that with a low nitrogen supply "potassium deficiency actually improves growth slightly" and results in more starch in the stems. Similarly, the writer's work with peas showed definitely less starch in those plants grown without potassium, but this was at a high level of nitrogen with both nitrate and ammonium salts present. Also, TURCHIN (**21**) believes that potassium is more essential for the utilization of ammonium salts than for nitrates. Hence, the results in the work with peas may be partially due to an excess of nitrogen in the ammonium form, and potassium may not be as important in the storage of starch as appeared at that time. WHITE (**24**), working with the tomato, concludes that "The observed effects of potassium deficiency on fruiting are those also associated with carbohydrate deficiency relative to nitrogen supply."

RICHARDS (**16**) believes that potassium is not essential to protein synthesis, but that it is necessary to maintenance of the protoplasmic complex.

Pertinent to the problem of the storage of carbohydrates is the suggestion of JAMES and CATTLE (**11**) that potassium is not part of the diastase molecule, but that it is likely to act as a catalyst in the building up of the diastase complex. Similarly, WHITE (**23**) believes that a major rôle of potassium is regulation of the carbohydrate metabolism through control of the starch-sugar balance by activation of amylolytic activity. It is quite possible that more information on this point might help to explain some of the discrepancies in the literature, especially those in which there is conflicting evidence as to the effect of potassium on the presence or storage of the different carbohydrates.

It seems highly probable that PHILLIPS, SMITH, and HEPLER (**14**) are correct in suggesting that "the function of the element in question may well be indirect."

Summary

Plants of tobacco were grown from seedlings in sand culture with a complete nutrient solution and with a similar solution in which potassium phosphate was replaced by sodium phosphate. Leaves were cut separately, and the fresh and dry weights were ascertained. Quantitative determinations of starch were made by the iodine precipitation method of PUCHER and VICKERY.

The control plants grew normally with more and larger leaves having greater fresh weight and dry weight than those deprived of potassium. The proportion of dry matter in the leaves was greater in the control plants

and decreased from the top toward the base for all plants, that is, decreased water content was found associated with potassium deficiency.

An apparent correlation between the supply of potassium and the capacity of the plant to store starch was noted, inasmuch as the maximal amount of starch in the leaves of the control plants in each series was greater than the maximum in the leaves of the plants deprived of potassium.

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RELATION OF pH TO GROWTH IN CITRUS

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(WITH TEN FIGURES)

Introduction

In a discussion of pH for healthy growth in citrus it was pointed out (**2**) that in culture solutions, growth was relatively poor at pH values above 8.0. For several years the problem of reaction and the growth of citrus has been studied under controlled conditions and in every case the conclusion has been reached that acidity is essential for prolonged health in the trees. A prerequisite, then, to healthy growth was the fact that during some period in the growth of the tree, it was necessary that acid conditions prevail.

Citrus, in California at least, is assumed to have a wide range of tolerance as regards pH. In contrast to the excellent groves found on soils as acid as pH 5.4 in Florida (**1**), it is concluded that equally fine groves in California may be found on soils with pH values of 8.5 or higher. According to certain recommendations, it is desirable to alter the pH of a soil only when the pH value exceeds 8.5 or is less than 5.0.

The degree of acidity or alkalinity of soils, such as is commonly encountered, is considered unimportant as regards any direct effect on the growth of citrus. The view is also frequently taken that pH determinations in solution cultures have little or no direct bearing on soil analysis or the growth of citrus in orchards.

In view of the wide divergence in pH values found in healthy groves in Florida and in California, it is difficult to reconcile the conclusions drawn from solution cultures with those obtained in the field. At first glance, it appears that results obtained in controlled artificial solution cultures should be severely discounted or should be considered as not being applicable in the field.

It should be stated, however, that pH determinations as carried out in commercial laboratories in southern California are commonly made upon a water suspension of dried soil, the dilution ranging from 1 part of soil in 1 part of water to 1 part of soil in 10 parts of water, the 1 to 5 ratio predominating. The pH values of these extracts are then determined by indicator or electrical methods. The use of rapid field tests in citrus groves has not met with much favor, largely because of the lack of experience in conducting such tests.

In solution cultures, the pH is determined by the indicator or electrical methods and the determination is simple and accurate. In soil cultures or under field conditions, the determination of pH has left much to be desired. Recently, the use of the speartype electrode (**6**) has brought greater ac-

curacy in the determination of pH in soils and as a result it becomes possible to compare more favorably the results obtained in solution cultures with those obtained in soil cultures or in the field.

The purpose of this paper is to emphasize the importance of pH for healthy growth in citrus and to show the results obtained when certain ranges of pH were used in solution cultures. Various sources of nitrogen were employed, in conjunction with ranges of pH, in the growing of citrus in culture solutions. Equally important are the soil cultures with citrus, in which the pH of the soil and the sources of nitrogen are related to growth. These studies then permit comparisons of the pH requirements of citrus in solution cultures with those found in soil cultures. The pH of soils as they occur under field conditions in the orchard will be considered in another report. In this way, evidence will be produced from several sources to show that when citrus trees are healthy they are growing primarily in an acid rather than in an alkaline medium and that the degree of acidity at which growth is best, is more acid than is commonly believed.

Experimentation

Out-of-door solution cultures

Eureka lemon trees budded on Koethen sweet orange as stocks were obtained from a nursery and were planted bare-root in aerated culture solution in 105-liter capacity galvanized iron containers on February 2, 1938. The containers were coated on the inside with (Gilacoat) asphalt paint and on the outside with aluminum paint.

The culture solution (3) employed had the composition (p.p.m.): Na, 7; K, 185; Ca, 159; Mg, 54; Cl, 10; NO_3, 718; SO_4, 216; PO_4, 105; B, 0.5; Mn, 0.25; and Fe, 0.1. Distilled water was used and iron tartrate was frequently added. The solutions were renewed on April 27, 1938. On June 13, 1938, each culture received 96 p.p.m. Mg and 210 p.p.m. NO_3.

The pH was adjusted by means of dilute solutions of sulphuric or nitric acids, or calcium hydroxide. Two cultures were grown at pH 4.5, using sulphuric acid for adjusting the pH in one case and nitric acid in the other. Calcium hydroxide solution, when required, was used for either culture. Similarly, two other cultures were grown at pH 6.0. A third pair of cultures (49 and 50) received no pH adjustment.

Many leaves curled and fell. On February 14, 1939, the entire tree of culture no. 50 weighed 1256 gm. (2.8 lb.). The control trees were in poor condition (fig. 1). When the pH of such cultures is adjusted to 4.5, recovery is slow because of the loss of leaves and food reserves.

The lemon trees in the pH 6.0 cultures could not be distinguished in their general appearance from the trees in the control cultures. Whenever fruits were produced, they turned yellow at small sizes and were subject to fungus invasion.

TABLE I

pH OF CONTROL LEMON CULTURES AT VARIOUS TIMES

DATE	CONTROL CULTURES; NO ADJUSTMENT OF pH	
	49	50
1938	*pH*	*pH*
April 27	4.5	4.5
May 19	6.0	6.2
June 2	6.0	6.6
June 10	6.4	6.6
June 24	6.7	7.2
July 1	6.6	7.2
July 11	6.6	7.0
July 14	6.6	7.0
July 22	6.8	7.2
July 28	6.8	7.2
August 5	6.8	7.2
August 13	6.8	7.2
September 1	7.0	7.6
September 24	7.2	7.6
October 29	7.6	7.6
November 2	7.3	7.6
November 11	7.3	7.6
November 19	7.3	7.6
November 26	7.4	8.1
December 1	7.4	8.0
December 10	7.4	8.0
December 15	7.8	8.0
December 23	7.6	8.0
1939		
January 3	7.8	8.1
January 19	7.4	8.0
January 27	7.6	8.0
January 30	7.6	8.0

Sulphuric acid was preferred to nitric acid for ease in adjusting and maintaining pH values. The tree growth with sulphuric acid was not inferior to that in which nitric acid was used. The effect of adjusting the pH of two cultures to 4.5 was to bring about excellent growth (fig. 2) when observed on February 14, 1939. The weight of an entire tree grown at pH 4.5 was 3141 gm. (7 lbs.) or about 2.5 times that of the control. The leaves, flowers, and fruits were characteristic of healthy trees. The roots were in excellent condition (fig. 3). One tree from a pH 4.5 culture bore 35 dark green fruits averaging $2\frac{1}{8}'' \times 1\frac{1}{2}''$ while another tree grown at the same pH bore 50 dark green fruits averaging $2\frac{3}{4}'' \times 1\frac{3}{4}''$.

With a similar culture solution made up with tap water, large tanks of 1400-gallon capacity were used in which to grow valencia orange, navel orange, lemon, and grapefruit trees. The pH of the solutions at times increased from 4.5 to above pH 6.0. This was accompanied by marked chlorosis even though iron tartrate was generously supplied. More frequent

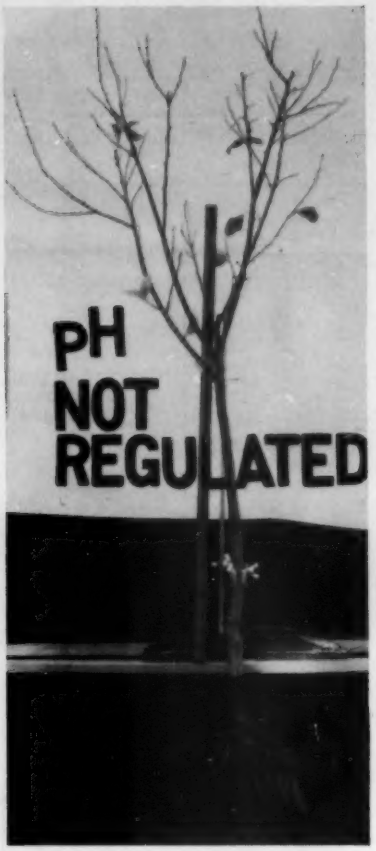

FIG. 1. Eureka lemon tree grown in an aerated culture solution with an initial pH of 4.5 and no subsequent pH adjustment.

adjustment of the pH was necessary to prevent chlorosis. A reduction in the pH of the culture solutions of chlorotic orange trees to pH 4.5 was soon followed by recovery to the green condition, even though many chlorotic leaves fell and weak twigs died. Difficulty is being had with lemon trees in restoring trees grown at or above pH 6.0 to health by reducing the pH to 4.5 because of the weak condition of the trees and loss of leaves.

SOLUTION CULTURES IN THE GLASSHOUSE

VALENCIA ORANGE CUTTINGS.—Experiments were begun March 15, 1937, with valencia orange cuttings in 12-gallon crocks of aerated culture solution made with distilled water. The basis of the culture solution was (p.p.m.): Na, 3.5; K, 71; Mg, 27; Cl, 5; SO_4, 196; B, 0.5; Mn, 0.25; Zn, 0.1; and Al (as aluminum citrate), 7.0. Every 18 liters of solution received 6.2

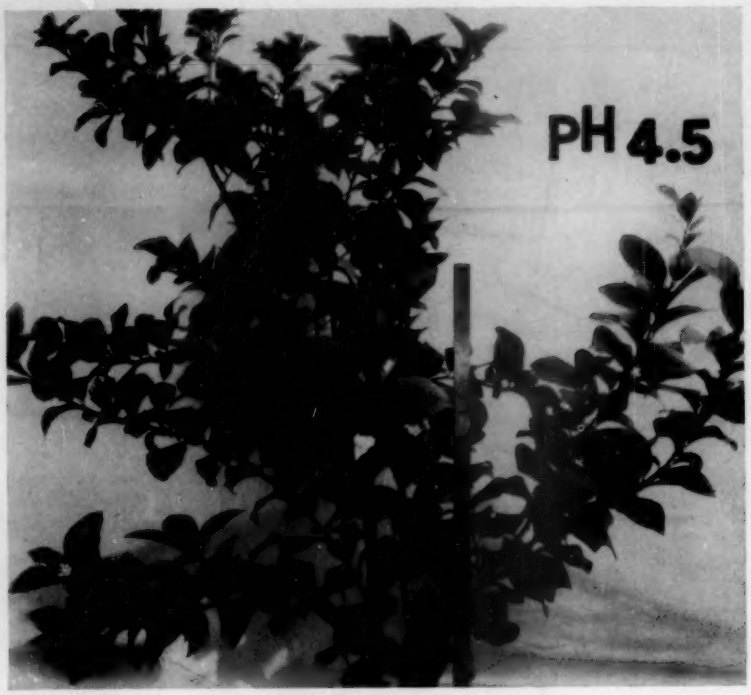

FIG. 2. Eureka lemon tree treated in every way like the tree shown in figure 1 except that the pH was adjusted to 4.5.

gm. calcium sulphate ($CaSO_4 \cdot 2H_2O$) or 2520 ml. of solution of distilled water saturated with calcium sulphate. Small additions of iron tartrate were made from time to time.

Each set of cultures consisted of 4 crocks. Two crocks received the phosphate only for a short period before the solutions were renewed, while the other 2 crocks received the phosphate at every solution renewal. The use of phosphate (KH_2PO_4) involved the addition of 52.5 p.p.m. PO_4 and 21 p.p.m. of K. The solution in one of each pair of crocks was adjusted to pH 4.5 and the others were adjusted to pH 6.0. Adjustments of pH were made twice every week with sulphuric acid or calcium hydroxide solutions.

Each set of 4 crocks received equal concentrations of nitrogen (122 p.p.m. N or 539 p.p.m. NO_3). The source of nitrogen was different in each set of cultures.

Every culture contained 6 valencia orange cuttings at the start. Replacements were very numerous in the cultures with phosphate continually present and in some of the cultures at pH 6.0 even with phosphate supplied at intervals.

FIG. 3. Root system of Eureka lemon tree shown in figure 2.

Table II presents data of interest in connection with young plants. When phosphate was continuously supplied, the young cuttings flowered and fruited heavily, but were unable to set any of the fruit, presumably on account of the low carbohydrate reserves. There was little or no vegetative growth as a result of this excessive fruiting. In other cultures in which large budded trees were used in the glasshouse (as with the lemon tree experiment previously described in this paper), phosphate continually present in the solution does not prevent excellent growth once the plants have reached an age suitable for bearing and maturing fruit. When of bearing age, it is advantageous to have phosphate present at all times.

So many replacements had to be made in some cases (table II) that either the cultures were abandoned or they were finally supplied with large cuttings grown in the same solution at pH 4.5 in which phosphate was present only at intervals. Even then the subsequent growth in many cases was poor. All of the trees were photographed on February 14, 1939, using approximately the same focal distance. In May, 1939, the same growth relationship held as on February 14 except on a higher level.

TABLE II

REPLACEMENT OF VALENCIA ORANGE CUTTINGS GROWN IN CULTURE SOLUTIONS

CROCK NO.	SOURCE OF NITROGEN	pH	PO_4 ALWAYS	REPLACED CUTTINGS	REMARKS ABOUT PLANTS IN CULTURES
					May 24, 1939
49	Calcium nitrate	4.5	No	0	Green
50	" "	6.0	No	10	Yellow
51	" "	4.5	Yes	9	Very poor, near dead
52	" "	6.0	Yes	7	
53	Ammonium nitrate	4.5	No	1	Green
54	" "	6.0	No	6	Green
55	" "	4.5	Yes	19	All dead
56	" "	6.0	Yes	6	Small and very yellow
29	Ammonium chloride	4.5	No	1	Green
30	" "	6.0	No	6	Yellow-green
31	" "	4.5	Yes	9	Small and yellow
32	" "	6.0	Yes	17	
33	Ammonium sulphate	4.5	No	2	Yellow-green
34	" "	6.0	No	8	
35	" "	4.5	Yes	26	Small
36	" "	6.0	Yes	20	
37	Sodium nitrate	4.5	No	1	Leaf burn at times
38	" "	6.0	No	16	All dead
39	" "	4.5	Yes	9	Very poor
40	" "	6.0	Yes	9	All dead
41	Magnesium nitrate	4.5	No	2	Yellow-green
42	" "	6.0	No	12	All dead
43	" "	4.5	Yes	6	
44	" "	6.0	Yes	9	
45	Potassium nitrate	4.5	No	4	Yellow-green
46	" "	6.0	No	6	
47	" "	4.5	Yes	11	All dead
48	" "	6.0	Yes	8	

Figure 4 shows the comparative growth of cultures 49 and 50, (see table II) in which calcium nitrate was the source of nitrogen. The best growth was made at pH 4.5, in a culture in which phosphate was present only at intervals. At pH 6.0, the leaves were quite yellow. Tests of the solutions were occasionally made to ascertain whether the nitrate was greatly reduced.

Table III shows the pH values of the calcium nitrate culture solution (crock 49) whenever regulation to pH 4.5 was made. Observation of the growth made it appear that the shift toward higher pH values than 4.5 were largely made during the production of new leaves and that the low pH values occurred principally as the foliage was maturing. HAAS and HALMA (**5**) have shown that maturing citrus leaves contain increasingly large amounts of calcium. If the nitrate and other anions were not ab-

FIG. 4. Calcium nitrate as a source of nitrogen for valencia orange cuttings grown in culture solutions with phosphate not continuously supplied. Left, pH 6.0; right, pH 4.5.

TABLE III

pH OF THE CULTURE SOLUTION BEFORE READJUSTMENT TO A GIVEN pH; PHOSPHATE IN CULTURES AT INTERVALS

Date	Calcium nitrate culture 49, pH 4.5	Ammonium nitrate culture 53, pH 4.5	Date	Calcium nitrate culture 49, pH 4.5	Ammonium nitrate culture 53, pH 4.5	Ammonium nitrate culture 54, pH 6.0
1938	*pH*	*pH*	*1939*	*pH*	*pH*	*pH*
June 7	7.5	5.9	Jan. 11	7.0	4.5	
11	7.0	6.0	13	7.0	4.5	
14	7.1	5.0	17	7.0	3.0	
17	6.7	4.5	20	7.0	3.0	
21	6.8	4.0	24	6.0	.....	
24	6.6	4.5	31	6.8	4.5	
28	6.0	4.0	Feb. 3	6.5	4.5	
July 5	4.5	3.8	7	5.0	4.5	
8	3.8	3.8	10	5.0	4.5	
22	5.7	4.3	15	4.5	4.5	
Aug. 3	4.5	3.0	17	4.5	.....	
9	4.5	3.0	21	4.0	4.5	
12	4.2	4.0	24	4.0	4.2	
16	4.1	4.0	28	3.8	4.0	
19	4.1	3.8	Mar. 3	4.3	4.3	
23	4.0	3.8	7	4.0	3.8	
30	4.3	3.8	10	4.0	3.8	
Sept. 2	4.5	4.5	14	4.1	4.1	5.0
6	4.2	4.2	17	4.0	4.2	4.2
8	4.3	.....	22	.....	4.5	6.0
13	4.5	4.5	24	5.0	4.3	4.4
16	4.2	4.2	27	5.0	4.0	4.0
21	4.2	4.2	31	5.0	3.8	6.0
23	4.3	.....	Apr. 4	4.5	4.0	6.0
Oct. 4	4.5	6.0	8	4.5	.....	6.0
Nov. 1	6.0	3.8	12	4.2	4.0	6.0
8	4.2	4.5	14	4.2	4.0	6.0
15	4.5	4.5	18	4.3	4.2	6.0
18	4.3	4.2	21	4.2	5.5	4.5
23	4.5	4.3	25	4.0	4.8	4.4
25	4.2	4.3	28	4.1	4.2	4.2
28	4.3	4.3	May 2	4.0	4.2	4.2
Dec. 6	4.3	4.5	5	4.5	4.5	4.4
9	4.2	4.2	10	4.2	4.4	4.2
13	4.2	4.2	13	4.2	4.4	4.2
16	4.5	4.2	17	4.4	5.0	4.2
20	4.2	4.2	19	4.5	5.8	6.0
23	4.5	4.3	23	4.0	4.5	4.5

sorbed at the same rate as the calcium, the tendency would be to increase the acidity of the culture solution during the periods of heavy calcium intake.

The calcium nitrate culture no. 50 (adjusted to pH 6.0) was relatively easy to keep at that value, the tendency largely being to shift to slightly higher pH values. The effect of the higher pH (6.0) of the culture solution on the growth of the cuttings is very marked (fig. 4).

Figure 5 shows valencia orange cuttings grown in culture solutions adjusted to pH 4.5 and 6.0 when ammonium nitrate was the source of nitrogen and when phosphate was not continuously present in the solution. Growth at pH 4.5 was approximately the equal to that when calcium nitrate was used (fig. 4). At pH 6.0, the growth was greater and of better color than

FIG. 5. Ammonium nitrate as a source of nitrogen for valencia orange cuttings grown in culture solutions with phosphate not continuously supplied. Left, pH 6.0; right, pH 4.5.

that produced with calcium nitrate. Table III shows some of the pH values reached by the solutions before readjustments of pH were made. The calcium nitrate culture solution either remained at pH 6.0 or went slightly higher while the tendency for the ammonium nitrate culture solution, adjusted to pH 6.0, was to shift toward greater acidity. This gave the am-

monium nitrate culture an advantage over the calcium nitrate culture in making the greater and healthier growth in the culture intended to be held at pH 6.0. Despite the difficulties of pH maintenance, figure 5 shows that at pH 6.0 the ammonium nitrate culture made fairly good growth but not as much as at pH 4.5. At these same pH values with the phosphate continuously present, young cuttings did poorly as seen in table II.

Figure 6 shows the growth of cultures in which ammonium chloride or

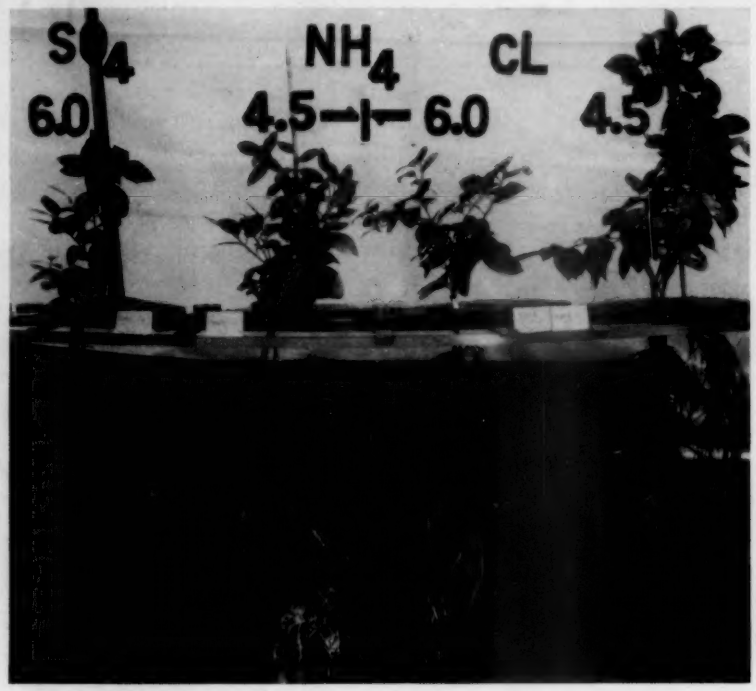

FIG. 6. Growth of valencia orange cuttings in solution cultures with ammonium sulphate or chloride as the source of nitrogen and with phosphate not continuously supplied. Right to left: ammonium chloride, pH 4.5 and 6.0; ammonium sulphate, pH 4.5 and 6.0.

sulphate was the source of nitrogen and in which phosphate was supplied at intervals. The plants at pH 4.5 did not make nearly as good growth as the ammonium nitrate or calcium nitrate cultures. The cuttings at pH 6.0 grew so poorly that cuttings were first grown in similar solutions at pH 4.5 and were then transferred to the pH 6.0 cultures.

Although the solutions at pH 4.5 tended toward lower pH values, the increasing acidity is not considered to be responsible for the smaller growth (fig. 6). It is more than likely that the enforced absorption of

ammonium as the sole source of nitrogen is injurious to the health of the plant. It is to be noted that no solution culture formula that makes use of ammonium nitrogen excludes the use of nitrate but rather makes the nitrate nitrogen predominantly greater than the ammonium nitrogen. Investigators admit using ammonium salts in small amounts in their culture solutions for the primary purpose of acidification or ease of pH maintenance.

It is desirable to interrupt the discussion at this point in order to report an experiment on the effect of increasing the ammonium nitrogen while keeping the total nitrogen constant. A series of three 48-liter crocks were used for aerated solution cultures in each of which three Eureka lemon cuttings were placed. The cultures have been growing more than a year, during which time the pH of the solutions were adjusted to 4.5. With increasing concentrations of ammonium sulphate there is greater difficulty in maintaining the pH of the culture solution at 4.5. The culture solution used in each crock had the following composition (p.p.m.): Na, 7; K, 185; Mg, 54; Cl, 10; NO_3, 225; SO_4, 216; PO_4, 105; B, 0.5; Mn, 0.25; and Fe, 0.1. Iron tartrate was supplied at intervals and distilled water was used throughout the experiment. No zinc or aluminum was used. To these culture solutions were added calcium nitrate and ammonium sulphate solutions as shown in table IV.

TABLE IV

SOURCE AND CONCENTRATION (P.P.M.) OF NITROGEN ADDED TO EUREKA LEMON SOLUTION CULTURES

	CALCIUM	NITROGEN (ADDED AS CALCIUM NITRATE)	SULPHATE	NITROGEN (ADDED AS AMMONIUM SULPHATE)
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Crock 26	298	209	48	14
Crock 27	279	195	95	28
Crock 28	238	167	190	56

Figure 7 shows the effect of increasing the ammonium nitrogen from 14 to 28 to 56 p.p.m., respectively, while adding sufficient nitrate to make the total nitrogen 223 p.p.m. As the ammonium nitrogen increased, the growth decreased. The total fresh weights of the trees and fruits were: 2444, 1758, and 1516 grams respectively, and the number of fruits were 12, 13, and 9, respectively, with increasing ammonium nitrogen. The enforced absorption of ammonium in the absence of sufficient nitrate does not appear to be beneficial in the growth of lemon cuttings.

Resuming our consideration of various sources of nitrogen at pH 4.5 and 6.0 with valencia orange cuttings continuously supplied with phosphate or supplied only at intervals, the study of sodium, potassium, and magnesium

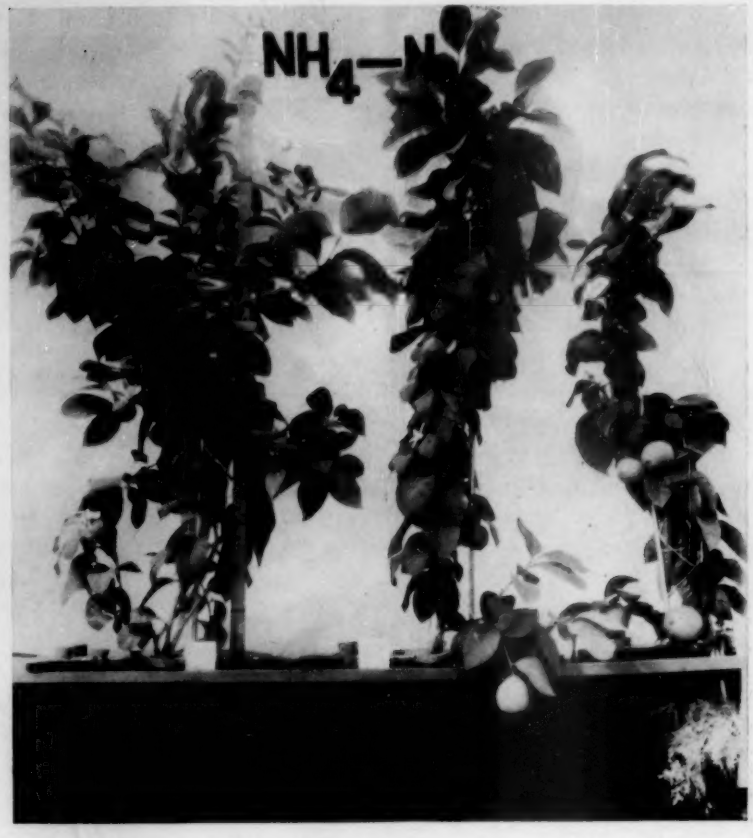

FIG. 7. Effect (in solution cultures of Eureka lemon cuttings) of increasing the ammonium nitrogen in the presence of a constant amount of total nitrogen. The pH was adjusted to 4.5. As the ammonium nitrogen was increased, growth decreased. Left to right: 14, 28, and 56 p.p.m. nitrogen.

nitrates as sources of nitrogen should be of interest. When these nitrates were used the growth of the cuttings was markedly inferior to that obtained when calcium or ammonium nitrate was used (fig. 8). Better growth was made at pH 4.5 than at 6.0 and when PO_4 was supplied at intervals rather than when continuously present. The leaves of the sodium nitrate culture burned upon becoming mature and upon becoming affected by accumulations of certain constituents in the leaves. The root systems were extremely long, with a scarcity of laterals.

The effect of pH on large-size valencia orange cuttings grown with phosphate continuously present was next studied because young citrus cuttings do not succeed very well when phosphate, in the concentration used,

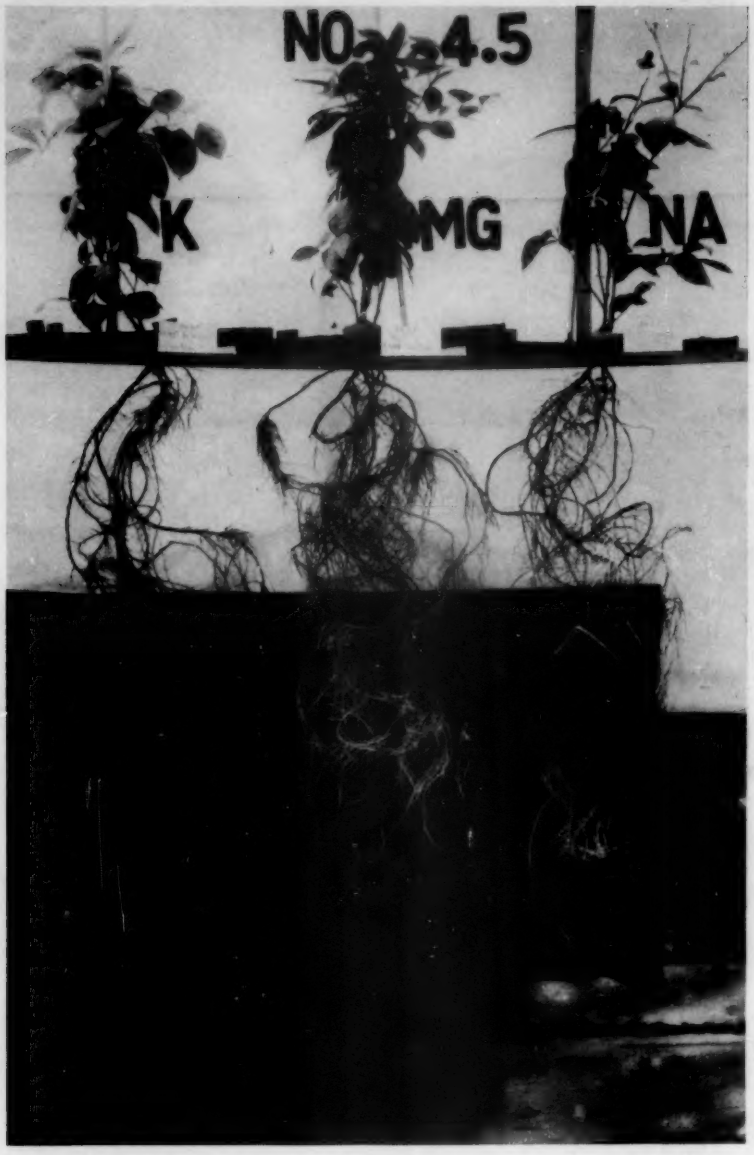

FIG. 8. Growth of valencia orange cuttings in culture solutions adjusted to pH 4.5 and with phosphate not continuously supplied. The sources of nitrogen, left to right: potassium, magnesium, and sodium nitrate.

is present continually in the culture solution. The young rooted cuttings were first grown in the same kind of solutions as later, except that in the young stages of growth the phosphate was present in the culture solution only at intervals and the pH was adjusted to 4.5. When a year or more in age, the cuttings were then supplied continuously with phosphate and the pH values of the solution were adjusted to 4.5 and 6.0 respectively. The largest plants purposely were used for the cultures later to be maintained at pH 6.0 in order to give them a good chance to make excellent growth.

It was found that at pH 6.0 with phosphate continuously present in the culture solution, the leaves were chlorotic while at pH 4.5 the leaves were green. The roots of the plants at pH 4.5 were considerably larger than at 6.0 despite the fact that when the differential pH values were begun, the plants used for the pH 6.0 cultures were superior to those used for the cultures at pH 4.5.

Soil cultures in glasshouse

Lemon cuttings in pH adjusted soil cultures.—River soil obtained near Riverside was used in 5-gallon tin containers coated with asphalt paint. One-half pound of dried blood was mixed thoroughly with the soil in each container. Small known aliquots of this soil were used to determine the amount of dilute sulphuric acid or sodium hydroxide necessary to add to the soil in order to secure various initial values when a ratio of 1 part of dry soil to 5 of distilled water was used in obtaining the soil extract. The pH of this soil suspension was determined with a glass electrode.

On December 27, 1938, the experiment was begun with 3 Lisbon lemon cuttings in the soils adjusted to various pH values. The cuttings at the lowest pH value had made one cycle of growth and part of the second cycle while the cuttings at the next higher pH value were just starting growth and none had started at the higher pH values. On February 20, 1939, the Lisbon lemon cuttings were discarded and 3 Eureka lemon cuttings were planted in each soil culture. By May 10, 1939, the culture that was begun at the lowest pH value had made the most growth; the culture begun at the second lowest pH value ranked second in growth, while in cultures begun at higher pH values, only 1 cutting put out a small shoot. The cuttings at the high pH levels failed to grow even though replants were used repeatedly. When such injured cuttings were planted in more acid soil, many recovered.

When these soils were first adjusted to definite pH values on a 1:5 soil-water ratio basis, the pH values of samples taken at the start of the experiment were: 4.18, 5.90, 8.05, 8.89, and 9.97, respectively. Determinations of pH by the 1:5 soil-water ratio method were made on soil samples taken and dried on May 2, 1939. The pH values were: 4.96, 5.60, 7.25, 7.27, and 7.81 respectively. Such changes in the pH of adjusted soils are common.

SHEAR (7) recognized this fact and waited 2 years for soils to attain their equilibrium. When the pH of the soil samples of May 2, 1939 was determined with the spear-type glass electrode (6), the values obtained were: 4.74, 4.93, 6.48, 6.62, and 6.74 respectively at moisture contents of 18.1, 18.0, 17.1, 13.0, and 21.1 per cent. respectively (dry soil basis). Growth at the lowest pH values was better than at the high pH values.

Before experiments with various sources of nitrogen in soil are discussed, reference should be made to the results (4) obtained with avocado seedlings grown in Hanford soil in asphalt-coated containers in the plant pathology temperature tanks maintained at 10°, 17°, and 24° C. respectively. In these temperature tanks the soil temperature is controlled while the temperature of the tops of the trees approximates that of the glasshouse. Two containers at each soil temperature received a calcium nitrate solution while two other containers at the same temperature received an ammonium sulphate solution. All containers thus received at the beginning of the experiment, equal amounts of nitrogen, either in the form of calcium nitrate or ammonium sulphate. Distilled water was used at all times. At the conclusion of the experiment, soil samples were taken from the containers and dried. pH determinations of water suspensions (1:5 soil-water ratio) were made and the results shown in table V were obtained.

TABLE V

EFFECT OF SOIL TEMPERATURE AND SOURCE OF NITROGEN ON THE pH OF (1:5 SOIL-WATER RATIO) SUSPENSIONS

SOIL TEMPERATURE	FERTILIZER USED (EQUIVALENT NITROGEN)			
	CULTURE NUMBER	CALCIUM NITRATE	CULTURE NUMBER	AMMONIUM SULPHATE
°C.		*pH*		*pH*
10	1	7.49	3	5.80
	2	7.48	4	5.92
17	5	7.49	7	5.61
	6	7.43	8	5.67
24	9	7.27	11	5.53
	10	7.49	12	5.49

The pH of the calcium nitrate-treated soil remained approximately the same at the various soil temperatures while that of the ammonium sulphate-treated soil was progressively lower with increasing soil temperature. Undoubtedly temperature affects the rate of nitrification of ammonium-nitrogen and the production of acid which lowers the pH. This fact should be

considered in growth studies that involve the use of ammonium sulphate at various soil temperatures.

EFFECT OF NITROGEN SOURCE ON GROWTH OF LEMON CUTTINGS.—In an experiment with equivalent amounts of nitrogen from various sources, small tin containers (6 inches in diameter × 6¾ inches high) coated with asphalt paint were used with about 3650 grams of air-dry pasture soil (Hanford series). 0.1825 gram of nitrogen was used to give a concentration of 50 p.p.m. of nitrogen (air-dry basis). A Lisbon lemon cutting was planted in each container on January 29, 1937. Distilled water was used at all times and the containers were without drainage. On February 25, 1937, the source of nitrogen had shown an effect (table VI) on the occurrence of new growth.

TABLE VI

SOURCE OF NITROGEN AND OCCURRENCE OF NEW GROWTH

SOIL TREATMENT	NUMBER OF CULTURES USED	CUTTINGS WITH NEW GROWTH
Control	9	0
Potassium nitrate	8	0
Calcium nitrate	8	1
Sodium nitrate	8	4
Magnesium nitrate	8	5
Ammonium nitrate	8	5
Ammonium chloride	8	4
Ammonium sulphate	8	8

The addition of certain forms of nitrogen to the soil caused a response in growth more quickly than when other forms of nitrogen were used. Potassium and calcium nitrate were the slowest in initiating new growth while ammonium sulphate was the most rapid. On March 31, 1937, the leaves of the ammonium sulphate-treated cuttings were the darkest green of all the cultures and had started their second cycle of growth before the others. On April 12, 1937, the ammonium sulphate cultures showed the most rapid growth. The sodium nitrate cultures were perhaps equivalent to the ammonium chloride cultures, the sodium and especially the chlorine being unfavorable. The slower action of nitrate nitrogen as compared with ammonium nitrogen is attributed by some investigators to the necessity of the plant first having to reduce the nitrate before such nitrogen is available for protein synthesis. In soil, it appears from the results obtained that the pH is a factor worthy of consideration in this difference in the rate of growth when nitrate or ammonium nitrogen is used.

The experiment was continued and on June 21, 1937, 50 p.p.m. of PO_4 as $Ca(H_2PO_4)_2 \cdot H_2O$, or 0.1825 grams P, was added to half of the cultures. On July 10, 1937, the phosphate treatment was repeated without any response being noted at any subsequent time.

The experiment was continued until December 14, 1937. Soil samples were taken and dried before terminating the cultures, the soil cores being taken the full depth of the containers. The pH values of (1:5 soil-water ratio) suspensions of these samples were determined with the glass electrode and the results are shown in table VII.

TABLE VII

EFFECT OF SOIL TREATMENT AND THE GROWTH OF LEMON CUTTINGS ON SOIL pH

FERTILIZER USED (EQUIVALENT NITROGEN)	pH OF (1:5 SOIL-WATER RATIO) SUSPENSION	
	PHOSPHATE FERTILIZATION	NO PHOSPHATE FERTILIZATION
	pH	*pH*
Control	6.58	6.61
Ammonium sulphate	5.92	5.95
Calcium nitrate	6.28	6.27
Potassium nitrate	6.37	6.18
Magnesium nitrate	6.30	6.42
Ammonium chloride	5.82	5.87
Ammonium nitrate	6.13	6.33
Sodium nitrate	6.49	6.58

Of the plants in the two most acid soils, the cuttings in ammonium-sulphate-treated soil were healthy while those in the ammonium-chloride-treated soil were injured by the chlorine. The cuttings in the ammonium-sulphate-treated soil made the most rapid and the healthiest growth of any of the nitrogen treatments.

INJURY AND RECOVERY OF LEMON CUTTINGS IN SOIL WITH VARIOUS SOURCES OF NITROGEN.—An experiment was conducted with lemon cuttings in containers of soil similar to those used previously. Each container was given 3844 gm. of air-dry soil which was a screened and well mixed lot of pasture soil of the Hanford series. A solution containing 0.1825 gm. of nitrogen was added to each culture (about 48 p.p.m. of nitrogen). Distilled water was used at all times.[1] The experiment was begun on April 20, 1937. The following sources of nitrogen were used: ammonium sulphate; ammonium sulphate commercial; calcium nitrate; calcium nitrate commercial; the nitrates of potassium, magnesium, sodium and ammonium; ammonium chloride; ammonium citrate; ammonium hydroxide; diammonium monohydrogen phosphate; and ammonium sulphate (in combination with 0.4 gram potassium sulphate per culture). An additional fertilizer application was made on July 9, 1937; on December 15, 1937, 2.5 times the usual application was made.

[1] Unless otherwise specified, C. P. chemicals, equivalent nitrogen, and distilled water were used and no drainage was provided. The cultures were protected at all times from rain damage.

In December, 1937, some of the cultures were given various amounts of N/10 sulphuric acid ranging from 1000 to 2500 ml. In a very short time practically all of these cultures were badly injured near the place of contact of the acid with the trunk and either died or were discarded before death. In such small containers it was not feasible to keep the strong acid from contacting and injuring the trunk.

On April 30, 1938, the remaining cultures differed in appearance, depending upon the source of nitrogen. Some cultures were declining while others, ammonium sulphate for example, were consistently healthy.

The cultures, except those treated with ammonium sulphate, were divided into certain groups for further treatment: 1. Cultures untreated, control. 2. Cultures watered with N/1000 sulphuric acid whenever water was required. 3. Cultures each received 2.5 grams of sulphur. 4. Cultures each received 5 grams of sulphur.

On August 10, 1938, pH determinations with the glass electrode were made by the 1:5 soil-water ratio method. Table VIII gives results ob-

TABLE VIII

EFFECT OF NITROGEN FERTILIZATION AND ACID OR SULPHUR TREATMENT ON THE pH OF (1:5 SOIL-WATER RATIO) SUSPENSONS

FERTILIZER TREATMENT (EQUIVALENT NITROGEN)	TREATMENT ON APRIL 30, 1938			
	NONE	2.5 GRAMS SULPHUR	5 GRAMS SULPHUR	N/1000 SULPHURIC ACID CONTINUALLY
	pH	*pH*	*pH*	*pH*
Control	7.01	----	6.47	----
Ammonium sulphate	4.29–4.91	----	----	----
Ammonium sulphate, commercial	4.35–4.70	----	----	----
Calcium nitrate	7.30	4.70	6.50	7.57
Calcium nitrate, commercial	7.06	5.48	5.10	7.23
Potassium nitrate	7.23–7.60	5.79–6.20	6.07	7.37
Magnesium nitrate	6.94–6.95	6.42	6.11	7.52
Sodium nitrate	8.37	5.29–6.00	5.90	8.16
Ammonium chloride	4.71	----	4.93	5.05
Ammonium nitrate	5.53	4.13	4.49	6.10
Ammonium citrate	6.09–6.41	4.81	4.01	6.74
Ammonium hydrate	6.10–6.41	4.35	----	6.40
Acid ammonium phosphate	5.44	4.50	4.42	5.43
Ammonium sulphate and potassium sulphate	4.12–4.67	----	----	----

tained. The ammonium-nitrogen fertilizers generally made the soil more acid while the tendency of the nitrate-nitrogen fertilizers was to make the soils slightly more alkaline. The larger the growth of the plant, the more distilled water (pH 5.2) was used. This was an aid in preventing a large increase in the pH of the soil. The use of very dilute sulphuric acid appears not to have reduced the pH of the soil as determined by the methods

TABLE IX

FRESH AND DRY WEIGHTS OF LEAVES, TWIGS, AND ROOTS OF LEMON CUTTINGS AS AFFECTED BY SOURCE OF NITROGEN AND pH OF THE SOIL MEDIUM

FERTILIZER TREATMENT (EQUIVALENT NITROGEN)	NO TREATMENT						2.5 GRAMS SULPHUR					
	LEAVES		TWIGS		ROOTS		LEAVES		TWIGS		ROOTS	
	FRESH WT.	DRY WT.	FRESH WT.	DRY WT.	FRESH WT.	DRY WT.	FRESH WT.	DRY WT.	FRESH WT.	DRY WT.	FRESH WT.	DRY WT.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	8.8	2.5	8.5	3.9	33.1	8.8	7.0	2.1	9.0	4.1	37.5	10.0
Ammonium sulphate	278.0	91.7	505.0	272.0	490.5	189.0	.....	.....	.....	.....	.....	.....
Ammonium sulphate, commercial	251.5	81.7	448.0	242.0	566.0	237.0	.....	.....	.....	.....	.....	.....
Calcium nitrate	15.0	4.0	17.0	8.0	25.3	9.0	60.2	19.0	40.7	16.5	51.5	15.0
Calcium nitrate, commercial	23.3	7.0	30.0	14.9	31.0	10.2	52.0	17.0	66.0	34.0	66.0	24.4
Potassium nitrate	26.5	7.0	34.5	15.0	36.0	11.4	61.0	18.0	65.5	31.8	69.7	23.9
Magnesium nitrate	18.3	5.3	21.7	10.2	30.5	9.3	54.0	17.7	39.0	19.1	60.2	18.0
Sodium nitrate	26.2	7.0	32.8	13.1	30.0	8.2	70.2	21.9	46.5	21.4	62.0	21.0
Ammonium chloride	4.0	1.3	4.9	3.0	.....	.....	.....	.....	.....	.....	.....	.....
Ammonium nitrate	21.5	6.0	25.1	11.0	35.4	10.0	50.0	16.0	34.0	15.3	53.0	16.6
Ammonium citrate	23.8	7.0	31.8	14.7	42.5	13.2	38.8	12.0	38.8	19.2	57.0	21.0
Ammonium hydroxide	25.5	8.0	27.0	13.7	35.7	11.1	37.8	12.0	47.5	23.0	68.0	23.9
Acid ammonium phosphate	18.7	5.4	28.4	15.0	41.7	13.2	56.3	18.5	50.2	23.5	65.7	22.0
Ammonium sulphate and potassium sulphate	255.5	80.4	448.3	238.2	516.5	207.0	.....	.....	.....	.....	.....	.....

TABLE IX.—(*Continued*)

Fertilizer treatment (equivalent nitrogen)	5 grams sulphur						N/1000 sulphuric acid continually					
	Leaves		Twigs		Roots		Leaves		Twigs		Roots	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	7.5	2.5	10.0	4.7	29.8	8.0	7.0	2.0	9.0	4.0	31.7	8.2
Ammonium sulphate	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ammonium sulphate, commercial	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Calcium nitrate	60.1	18.5	45.3	22.0	77.4	24.8	61.8	19.8	33.1	15.0	55.2	16.5
Calcium nitrate, commercial	72.0	23.0	70.0	34.4	75.5	26.1	54.7	19.0	52.1	25.4	64.7	20.5
Potassium nitrate	57.9	17.7	62.8	28.7	77.2	25.9	64.8	18.7	48.1	21.1	70.5	20.4
Magnesium nitrate	48.5	16.1	39.5	19.7	70.1	23.2	51.3	18.0	42.3	21.1	66.1	23.0
Sodium nitrate	55.0	16.9	46.0	21.9	56.5	18.2	49.2	15.0	40.3	18.1	48.0	15.0
Ammonium chloride	4.8	1.3	3.7	1.8	4.2	1.2	9.1	2.1	6.8	4.0	13.8	4.0
Ammonium nitrate	60.5	19.0	37.2	17.7	60.0	19.2	49.5	17.6	40.3	19.9	70.1	22.1
Ammonium citrate	28.7	8.2	39.2	19.2	63.0	18.5	39.4	12.6	45.5	23.0	71.1	22.0
Ammonium hydroxide	.....	.....	.....	.....	.....	.....	38.7	12.7	46.5	23.0	69.0	23.1
Acid ammonium phosphate	48.9	16.9	56.0	27.2	71.0	25.0	67.1	21.7	61.5	29.0	83.3	28.0
Ammonium sulphate and potassium sulphate	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

used. In sampling each soil culture, however, a composite or average was made of several cores taken to the bottom of the container and the pH of the upper soil may have been lower than that of the deeper soil. This at least is the condition met with commonly in the field.

When attempts were made to lower the pH of the entire mass of soil by the use of N/10 sulphuric acid, the concentration of acid used proved injurious when in contact with the trunk in these small containers. Attempts were made to ridge the soil about the tree and prevent the contact but it was not feasible in the small size container.

When sulphur was used instead of N/10 sulphuric acid, no difficulties resulted from concentrations of acid against the trunk. The formation of acidity is gradual enough and moves gradually downward, with no apparent result except in the ease of water penetration. There is considerable evidence in the marked response of the cuttings to the use of sulphur or dilute sulphuric acid to indicate that lemon cuttings grow better under more acid soil conditions than is usually considered most favorable.

The data in table IX gives the weights of the lemon cuttings in the various cultures at the end of the experiment. The cultures were photographed August 29, 1939, and show the effectiveness of the acidifying treatments of April 30, 1938. Figure 9 is typical of the results obtained

FIG. 9. Lemon cuttings grown in Hanford soil that received calcium or potassium nitrates as the source of nitrogen. Injury resulted but recovery was brought about by the use of sulphur or sulphuric acid.

and shows the marked benefit to lemon cuttings in nitrogen-fertilized Hanford soil in which the soil pH is taken into consideration. When nitrogen was not supplied to the cultures, acid treatment gave very little improvement because of the limitations on growth. These studies have thrown light on one phase of the mode of action of copper sulphate applications in soil upon the growth response by citrus cuttings.

COPPER SULPHATE EFFECTIVENESS IN RELATION TO pH IN LEMON CULTURES.—An experiment was begun with undrained containers in which was placed 4000 gm. (air-dry basis) of Hanford soil from the same lot previously used. A solution of copper sulphate was added to the soil cultures as shown in table X. The soil in each container was thoroughly mixed after the addition of copper sulphate. On March 8, 1938, a Eureka lemon cutting was planted in each container of soil. 0.1825 gm. of nitrogen in solution was added to each culture. Distilled water was used at all times.

Ammonium sulphate was used as the source of nitrogen in one series of cultures while calcium nitrate was used as the source of nitrogen in the other series. 0.3650 gm. of nitrogen was added August 12, 1938, and again on December 19, 1938, to the cultures of each series.

In January, 1939, the leaves of the calcium-nitrate series were turning yellowish green and were being lost while in the ammonium-sulphate series the leaves were dark green and securely attached. On April 5, 1939, the weights of the cuttings were determined (table X). Composite cores of soil were taken at that time from each culture to the full depth of the container for pH determination with the spear-type glass electrode.

The calcium-nitrate cultures had 60 immature leaves of 48 gm. fresh weight while the ammonium sulphate cultures had 286 immature leaves of 241 gm. fresh weight and in addition 4 lemon fruits $\frac{1}{4}''$–$1''$ in size.

Table X shows that as the copper sulphate concentration was increased in the calcium nitrate series, fewer leaves were lost. High concentrations of copper depressed growth. There was little if any benefit from the use of copper sulphate in the ammonium-sulphate series, but the benefit in the calcium-nitrate series was pronounced.

Table XI reveals that the use of copper sulphate in most cases assisted in lowering the pH of the soil in the calcium-nitrate series. The pH determinations were made on fresh composites at the soil moisture content and on the dried soil by the 1:5 soil-water ratio method. Table XI shows that when the pH values were lowered sufficiently, the cuttings appeared healthy. These results were obtained with the same lot of soil in which acidification by means of sulphur or sulphuric acid also was beneficial. If copper is deficient in cuttings in the calcium-nitrate series it must be the result of high soil pH because sulphur, sulphuric acid, or ammonium sulphate corrected the difficulty without the addition of copper. In ex-

TABLE X

GROWTH OF EUREKA LEMON CUTTINGS AS AFFECTED BY VARIOUS CONCENTRATIONS OF COPPER SULPHATE WITH EQUIVALENT NITROGEN FROM CALCIUM NITRATE OR AMMONIUM SULPHATE SOURCE

Copper added to air-dry soil	Mature leaves						Twigs				Roots			
	Calcium nitrate			Ammonium sulphate			Calcium nitrate		Ammonium sulphate		Calcium nitrate		Ammonium sulphate	
	No. of leaves	Fresh wt.	Dry wt.	No. of leaves	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
p.p.m.		*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0.5	0	.....	.....	79	47	15.5	14	5.7	45	21.1	10	3.0	74	23.7
1.0	0	.....	.....	73	64	21.5	8	3.3	57	30.2	10	2.7	68	23.5
2.5	6	5.0	.....	64	52	17.0	11	5.0	50	23.9	15	4.3	74	20.7
5.0	2	.....	.....	82	53	17.8	14	6.5	52	23.7	17	5.3	65	20.0
7.5	16	18.0	5.0	86	47	15.9	32	15.1	32	15.4	48	15.0	54	18.0
10.0	18	13.0	4.9	79	48	17.4	22	9.8	52	26.5	25	7.8	67	24.9
15.0	41	25.0	9.0	89	53	18.1	18	8.3	42	22.6	26	8.0	61	23.3
20.0	50	36.0	12.1	81	45	15.5	33	15.0	30	13.9	47	18.1	50	15.7
30.0	29	19.5	6.5	53	45	14.8	18	7.5	47	23.7	19	4.5	69	26.9
40.0	60	44.0	15.3	86	49	15.8	45	21.3	38	20.0	82	32.7	48	18.0
50.0	53	42.0	13.7	74	40	14.7	30	14.0	46	21.1	41	12.7	76	22.7
75.0	23	12.0	4.0	64	36	11.9	8	4.0	38	17.8	13	4.0	60	23.9
100.0	8	5.0	1.0	52	30	10.0	5	2.3	17	7.2	8	2.3	34	10.5
150.0	20	9.0	2.9	.....	.....	.....	9	4.2	.....	.....	14	4.5	.....	.....
0.0	18	22.0	.....	80	45	15.4	19	8.8	49	24.7	30	8.7	61	20.0
0.0	25		.....	74	50	17.4	17	7.8	43	22.8	27	7.4	68	22.4
0.0	3		.....	74	50	17.0	22	9.1	53	28.8	18	4.9	71	28.5
0.0	0		.....	70	42	15.0	23	10.3	50	27.7	22	6.7	64	22.9

TABLE XI

EFFECT OF SOURCE OF NITROGEN, PLANT GROWTH, AND COPPER SULPHATE TREATMENT ON pH OF SOIL CULTURES

Copper added to air-dry soil	Calcium nitrate series			Ammonium sulphate series		
	pH at soil moisture content	Soil moisture	pH of 1:5 soil-water suspension	pH at soil moisture content	Soil moisture	pH of 1:5 soil-water suspension
p.p.m.	*pH*	%	*pH*	*pH*	%	*pH*
0.5	6.10	7.87	6.71	4.52	7.46	4.70
1.0	6.47	10.26	6.93	4.72	8.38	5.27
2.5	6.14	9.00	6.63	4.00	4.32	4.47
5.0	6.00	9.84	6.63	4.31	9.55	4.81
7.5	6.47	10.81	7.11	4.17	6.15	4.74
10.0	5.96	8.55	6.74	4.11	7.46	4.58
15.0	6.03	5.51	6.90	4.33	9.23	4.94
20.0	5.91	9.10	6.76	5.00	9.52	5.46
30.0	6.44	12.07	6.96	4.37	7.07	4.75
40.0	6.08	9.45	6.64	4.33	5.50	4.81
50.0	6.30	9.73	6.82	4.11	10.18	4.60
75.0	6.22	10.73	6.93	4.02	11.38	4.60
100.0	6.10	10.64	6.81	4.34	10.60	4.87
150.0	5.91	7.89	6.57	4.57	12.22	5.34
0.0	6.49	9.33	7.14	4.42	8.82	4.90
0.0	6.48	7.45	7.24	4.16	5.76	4.60
0.0	6.47	10.12	7.05	4.02	8.49	4.49
0.0	6.78	12.40	7.20	4.09	4.98	4.64

periments involving the use of copper sulphate in soil it is essential that the pH factor be controlled because the changes in pH may be of greater significance than the changes in the actual copper concentration in certain soils.

Many experiments have proved the more rapid growth of lemon cuttings in Hanford soil when ammonium sulphate was used rather than an equivalent amount of nitrogen in the form of calcium nitrate. On January 28, 1938, 3773 gm. (air-dry) Hanford soil was used in containers 6″ diam. × 6¾″ deep without drainage. A lemon cutting was planted in each culture and 0.365 gm. of nitrogen as calcium nitrate or ammonium sulphate was added. On December 7, 1938, the average twig length per culture in the ammonium-sulphate series was 48 inches and 40.3 inches per culture in the calcium-nitrate series. The leaves in the calcium-nitrate series were in poor health as compared with those in the ammonium-sulphate series.

Hydrogen ion determinations of composite dried cores of soil taken to the depth of the containers were: 6.90, 7.15, 7.18, and 7.36 for the calcium-nitrate series, and 4.94, 5.33, 5.35, and 5.71 for the ammonium-sulphate series. The cuttings in the best condition were those in the more acid series. Later, with lemon cultures grown out-of-doors, it will be shown that the acidity rather than the nature of the nitrogen is important for growth of lemon trees.

LEMON CULTURES CONTAINING TOXIC AMOUNTS OF BORON.—On February 4, 1938, 2-gallon crocks without drainage were used as containers for Hanford soil (9675 gm. air-dry) that was well mixed after receiving a solution containing 0.1014 gm. of boric acid. A Eureka lemon cutting was planted in each container. Subsequently, the cultures of one series each received (only initially) 15 ml. of dilute nitric acid solution (2 ml. concentrated nitric acid in 8 liters distilled water) as the source of nitrogen while those of the other series received an initial application of calcium nitrate solution containing 0.5 gm. of nitrogen.

It was noted that the cuttings in the nitric acid series grew rapidly but the leaves showed serious boron-excess symptoms with the burning and loss of many leaves. The cuttings in the calcium nitrate series showed only minor boron-excess symptoms and the cuttings appeared extremely healthy until the leaves began to turn yellowish green. The leaves in the nitric acid cultures also became yellowish green and showed very little if any boron injury in new leaves. The pH of (1:5 soil-water ratio) suspensions of the dried composite soil cores taken the full depth of each culture showed a pH range of 7.23–7.40 for the nitric-acid series and 6.93–7.20 for the calcium-nitrate series. Excessive initial acidity no doubt brought about boron injury because of high boron availability while calcium nitrate was useful in preventing boron injury. When the pH in the soil in each series of cultures became sufficiently high, boron-excess ceased to be a problem while the OH concentration became important. When boron is a factor in soil, a range of pH may be found in soil in which excellent growth may be obtained with the least boron injury.

In the present experiment, the addition of 40 p.p.m. copper as copper sulphate furnished sufficient acidity to cause a thorough greening-up of yellow leaves within less than one week with no evidence of boron-excess symptoms in the calcium-nitrate series. The calcium-nitrate cultures without the copper sulphate addition had a pH of 6.47 at a soil moisture content of 8.96 per cent. (dry-soil basis) with the spear-type glass electrode and 7.14 by the 1:5 soil-water ratio method. The calcium-nitrate cultures to which 40 p.p.m. copper as copper sulphate had been added, showed pH values about 6.00 at the soil moisture content of 6.34 per cent. and 6.94 by the 1:5 soil-water ratio method. Other pH determinations on calcium-nitrate cultures not copper-sulphate treated were 6.46, 6.49, and 6.45 respectively at the soil moisture content, and 7.34, 7.28, and 7.18 respectively at the 1:5 soil-water ratio. The copper sulphate solution was applied to the surface of the soil while the soil sample cores were taken the full depth of the containers. It is reasonable to conclude that the pH was lower near the surface of the soil than at the lower depths. The pH change brought about in soil when copper sulphate is added, may explain in part the response frequently found in some soils and not in others.

The effects of pH changes in soil on the growth of valencia orange cuttings is not as evident as with lemon cuttings but are equally definite.

VALENCIA ORANGE CUTTINGS.—On March 20, 1937, asphalt-coated tin containers 6″ diameter × 6¾″ high and without drainage, were given 4220 gm. (air-dry) of screened and well-mixed Hanford soil of the same lot as was previously used. Whenever nitrogen was applied, equivalent amounts (0.1825 gm.) were used from the following sources: calcium nitrate, ammonium sulphate, potassium nitrate, diammonium hydrogen phosphate, and ammonium citrate. Untreated control cultures also were used.

The application of large amounts of N/10 H_2SO_4 to certain of the soil cultures caused gum formation and subsequent death of the tops because of contact with the trunk bark, hence N/1000 H_2SO_4 was used in later treatments of these nitrogen cultures.

By June 8, 1937, the ammonium-nitrogen cultures had shown considerably more rapid growth than the nitrate-nitrogen cultures. On August 10, 1938, the untreated cultures were yellow and, while not making much if any new growth, were able to retain their leaves.

The calcium- or potassium-treated cultures made fair growth for a time and then were unable to retain many of the old leaves. In some cases the new leaves produced were severely mottled and in many cases the leaves were short and extremely narrow. The cultures treated with ammonium sulphate were the best and became so large that it was a problem to keep the leaves from wilting. The ammonium phosphate or citrate cultures were far superior to the nitrate cultures but were scarcely the equal of the ammonium sulphate cultures.

On August 28, 1938, core samples of soil were taken to the depth of the containers and the samples were composited for any one container, dried, screened, and mixed. The pH of the 5 or more cultures in each nitrogen series was determined by the glass electrode in (1:5 soil-water ratio) suspensions and the following ranges of values were found: Untreated, control cultures, pH 6.84–6.92; calcium nitrate, 7.30–7.73; potassium nitrate, 7.13–7.92; ammonium citrate, 5.78–6.10; diammonium hydrogen phosphate, 5.26–5.45; and ammonium sulphate, 4.49–5.00. The pH of the soil was found to be related to the growth produced.

VALENCIA ORANGE INJURY AND RECOVERY.—The photograph (fig. 10) taken February 14, 1939, illustrates the beneficial effects of increased acidity under the conditions of the experiment. The three cultures (fig. 10) were affected most by the continued use of calcium nitrate in the experiment just described and had pH values above 7.0. The cuttings were similar in appearance when selected for the recovery experiment. The two large cuttings to the left in figure 10 show the improvement in growth when N/500 sulphuric acid was used for irrigating the cultures as compared with distilled water alone.

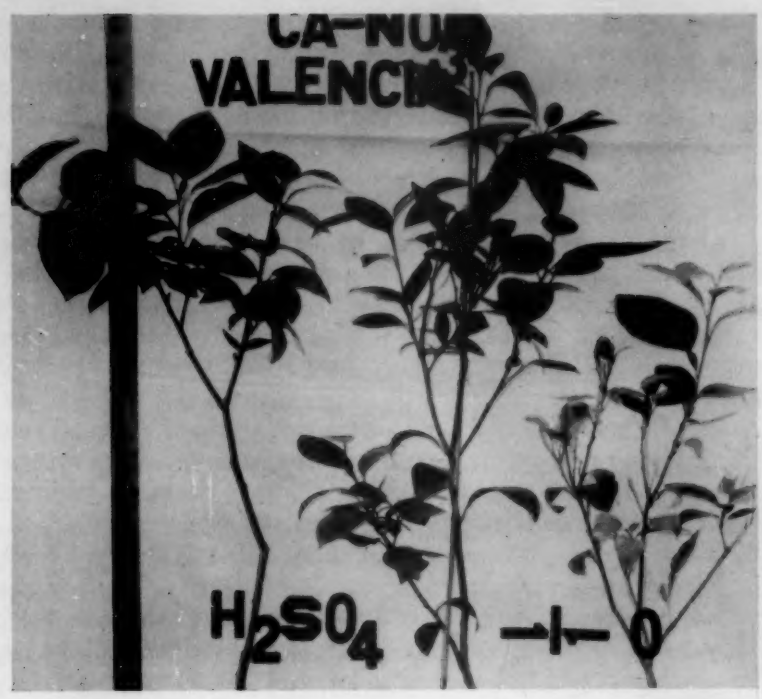

FIG. 10. Valencia orange cuttings grown in Hanford soil that received calcium nitrate as the source of nitrogen. Right, no additional treatment; 2 cultures on left, dilute sulphuric acid $\left(\frac{N}{500}\right)$.

The leaf area of some of the valencia orange cultures was measured in April, 1939. A culture with potassium nitrate as the source of nitrogen had a leaf area of 54.1 square inches; another with calcium nitrate as the nitrogen source, had a leaf area of 117.7 square inches. A culture that received calcium nitrate and later some sulphur had a leaf area of 199.5 square inches; another that received calcium nitrate and later some sulphur and dilute sulphuric acid had a leaf area of 286.9 square inches. These represent only a few of the many cultures grown that show good recovery upon lowering the pH of the soil.

The experiments cited thus far stress the importance of acidity in the growth of citrus. It has always been a question as to the source of the markedly beneficial action of ammonium sulphate in soil upon the growth of citrus. Experiments conducted out-of-doors with lemon cuttings in soil cultures, give additional evidence that the acidity produced by ammonium sulphate in soil is of value in addition to the nitrogen factor.

Soil cultures out-of-doors

Lemon cuttings.—Out-of-doors, on a rain protected table in the lath enclosure, lemon cuttings were grown in asphalt-coated tin containers 6″ diameter × 6¾″ high, without drainage. A 4000 gm. sample (3939 gm. air-dry) of screened and well-mixed pasture soil, from the same lot previously used, was added to each container. A Lisbon lemon cutting was planted in the soil of each container on July 23, 1937. The cultures, except the controls, received 0.365 gm. of nitrogen as ammonium sulphate, potassium nitrate, or calcium nitrate. To 8 of the 16 cultures in each series at the start of the experiment, an amount of sulphuric acid was added in order to supply as much sulphate as occurs in the amount of ammonium sulphate used. No additional acid was used to equal the nitric acid that results from the transformation of the ammonium into nitric nitrogen.

On November 22, 1938, the lengths of the twigs were measured and it was found that the average total twig length of the 8 controls (checks) that received no nitrogen, was 29.2 inches while that for 8 other controls (checks) that received no nitrogen but received 251 ml. of 0.1046 N sulphuric acid at the start of the experiment was 33.9 inches. Similarly, the 8 cultures that received calcium nitrate but no acid had an average total twig length of 59.9 inches, while the average for the cultures that received acid was 64.5 inches. The 8 cultures that received potassium nitrate had an average total twig length of 59.9 inches while the average when acid was used, was 73 inches. This last value compares favorably with 71 inches average total twig length when ammonium sulphate was used without the addition of acid.

Other experiments are being carried out in this direction but they have not been operating sufficiently long to serve the present needs. The experiment just described, makes it appear that much of the value of ammonium sulphate over that of calcium or potassium nitrate in citrus growing may reside in the acid residues. Where alkaline residues can be reduced or readily leached away, calcium or potassium nitrates approach ammonium sulphate in value although leaching is necessarily wasteful of nitrogen.

Since lack of drainage in these containers prevents the removal of the bases, it is essential that acid be added to the cultures. Drainage was avoided in order to prevent losses of nitrogen which might affect growth. Likewise, all cultures were protected from rain in order not to saturate the soil and not to lose or gain nitrogen through splashing of rain against the soil.

From the experiments described, it is seen that the pH that favorably affects the growth of citrus is much lower than is ordinarily assumed. The determinations of pH under field moisture conditions, give a lower range of pH values than those made by the conventional (1:5 soil-water ratio)

method and give more nearly the actual pH of the soil in which growth takes place. The pH values of soils arrived at with the use of the spear-type glass electrode allows greater emphasis to be given to the results obtained with solution cultures. Determinations of the pH of soils at moisture contents above the soil moisture content in the field have caused a misunderstanding of the pH requirements of these trees and a lack of appreciation for results found (2) through the use of solution cultures.

Summary

The growth of citrus and other subtropical trees in artificial solution cultures was found to be affected by the pH of the medium in which the roots grow. The trees, while tolerating extremely low or high pH values in their nutrient medium, made greater and healthier growth in acid than in alkaline solutions. The degree of acidity at which growth is best, is more acid than is commonly believed.

The conception that these trees grow well at pH values as high as 8.5 or higher is based on faulty methods of preparing soil samples for pH determination. The pH of soils, as will be further elaborated upon in another report, depends upon the soil moisture content as well as on other factors and may vary considerably according to the soil moisture present at the time the determination is made.

When determinations of pH are properly made, it is found that the results obtained with artificial solution cultures as well as with soil cultures, agree very well with those obtained in the field.

The importance of pH for healthy growth has been emphasized. Several sources of nitrogen have been used together with various pH ranges in solution cultures. Equally important are the soil cultures in which the pH of the soil and its relation to the source of nitrogen has been determined.

The conclusion is reached that although trees in California are grown in the field under an irrigated agriculture, they are growing primarily in an acid rather than in an alkaline medium, and that the acidity of the medium is far greater than has previously been assumed.

Thanks are due Professor M. R. Huberty and Mr. W. E. Picker of the Division of Irrigation for suggestions regarding the plant-water relations and other phases of soils. Thanks are also due Mr. J. S. Millar, who assisted in the daily routine of the cultures.

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THE BALANCE SHEET OF METABOLITES FOR POTATO DISCS SHOWING THE EFFECT OF SALTS AND DISSOLVED OXYGEN ON METABOLISM AT 23° C.[1]

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(WITH TWO FIGURES)

Introduction

This paper presents a summary of the effects of various salt and oxygen treatments so chosen that they comprise a range of conditions of peculiar interest to the student of salt accumulation.

In any account of plant metabolism important processes may evade recognition unless a balance sheet of the principal metabolites can be prepared. Such balance sheets have rarely been attempted although the work of Raistrick *et al.* (**31**) on fungi is a notable exception. The work of Raistrick was concerned, however, with the use of organisms under standardized nutritional conditions to effect organic synthesis and transformations; it did not describe the effect on the metabolism of a given organism of a range of external and nutritional conditions. There is need of more attention to balance sheets of plant metabolism and especially so with reference to the vexed problems of respiration. Deviations from the familiar equations $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 674{,}000$ cal. and $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 25{,}000$ cal. often tacitly accepted as representative of plant respiration, are multiplying. The work of Gane and others (**11, 16, 17**) has demonstrated the production of volatile substances other than carbon dioxide and large discrepancies between respiration and the simple equations above may be encountered. For example, Archbold and Barter (**1**) observed that the loss of sugar and acid in respiring apples exceeded by 17 to 30 per cent. the carbon dioxide evolved. On the other hand, Dastur and Desai (**10**) found that in the respiration of rice the carbon dioxide evolved exceeded the utilization of sugar and they believe that acids formed during the synthesis of protein form the source of the carbon involved. Again Lundegårdh (**23**) states that in excised wheat roots 30 to 60 per cent. more glucose disappears than is accounted for by respiration and he tacitly assumes that the discrepancy is a measure of the sugar used in growth. In experiments with excised roots Hoagland has observed cases in which a very rapid disappearance of sugar occurred and carbon dioxide production continued after the cells seemed to be free of sugar.[2]

[1] This is the second of a series of papers on The Biochemistry of Salt Absorption by Plants. The authors are indebted to Prof. Hoagland for proof-reading this paper.

[2] Results privately communicated.

From our present standpoint the outstanding fault in the above equations is lack of any implication that respiration concerns substances other than carbohydrates or that aerobic respiration and nitrogen metabolism are mutually dependent processes in plants.

The variation between replicate samples of standard potato discs is small (**47**) and the effect of metabolism on the constituents of the tissue can be obtained by difference between the composition of the initial and final, experimentally treated, batches of discs. It is possible, therefore, to draw up a balance sheet with reference to any metabolite, *e.g.*, carbohydrate, the fate of which is known with sufficient certainty so that its products can be determined. Balance sheets for dry-weight, calorific value, and total carbon may be similarly constructed and discrepancies in them would indicate an incomplete knowledge of the metabolic processes of the cells. Confidence that the effect of the various treatments on the metabolic processes of potato discs had been adequately comprehended was gained only when these various balance sheets appeared in mutual agreement and no longer showed discrepancies greater than the legitimate but small errors of the measurements. The balance sheets demand analytical data which are absolute and not merely of relative value. The methods already outlined (**47**) were carefully scrutinized and standardized. The concordant results from many different determinations obtained by the combined efforts of different individuals testify to the accuracy which has been attained.

Methods

Analytical procedures

The analytical procedures used in this paper and not hitherto referred to were the determination by wet combustion methods of the total carbon content of vacuum dry potato samples; the determination of the total uronic acid content of similar samples; and the measurement after their combustion to carbon dioxide of the volatile organic compounds produced by metabolizing potato discs. The wet combustions were carried out on re-dried samples (approximately 100 mg.) by a method used for determination of carbon in soils.[3] The method described by Raistrick (**31**) has also been used successfully, especially in connection with the removal of carbon from the discs by blotting. The uronic acid determinations were made by the method described by Dore in which the uronic acid is distilled with HCl and produces furfural plus carbon dioxide. The sample was heated under reflux condenser with 12 per cent. HCl for 4.5 hours. The carbon dioxide evolved in heating was absorbed in a potash bulb (after purification in the train of absorbents recommended by Dore) and deter-

[3] The addition of mercuric oxide was found to prevent the distillation of free bromine from the oxidation of tissue which contained bromide.

mined by weighing. The pectin value of the uronic acid found could be calculated knowing that each molecule of carbon dioxide collected corresponded to one molecule of uronic acid and assuming that this formed part of a tetragalacturonic-galactose-arabinose complex or its methyl esters (**7, 12, 13**). The volatile substances other than carbon dioxide which may be produced during the respiration of plants have recently received considerable attention. Such compounds were converted to carbon dioxide by passing the suspected gases through a heated combustion tube charged as in standard combustion practice with copper oxide (pure, from wire) and lead chromate.

The carbon equivalent of the volatile substances was obtained from the yield of carbon dioxide which was determined either volumetrically or by absorption in standard potash bulbs (pattern after CLASSEN) according to convenience. The variations in this basic procedure which were made to meet the needs of special experiments are described in the appropriate place in the text.

TREATMENTS OF THE POTATO DISCS

The general effect of salts and aeration on the metabolism of thin discs of potato tissue is shown by the effect of six different treatments as follows:

(1) Discs in distilled water at 23° C., aerated by a rapid stream of carbon-dioxide-free air.

(2) Discs in aerated dilute potassium bromide solution at 23° C. and of a strength (0.005 equiv. per liter) such that accumulation of the salt by the tissue would definitely occur.

(3) Discs in a relatively strong potassium bromide solution (0.05 equiv. per liter) aerated with carbon-dioxide-free air and at 23° C.

(4) Discs in dilute potassium bromide solution (0.005 equiv. per liter) at 23° C. but aerated with an oxygen-nitrogen mixture rich enough in oxygen (3.80 per cent.) to prevent break-down in the tissue, but too poor in oxygen to permit conspicuous salt accumulation.

(5) Discs in dilute potassium nitrate solution (0.005 equiv. per liter) aerated with carbon-dioxide-free air and at 23° C.

(6) Discs in dilute calcium bromide solution (0.005 equiv. per liter) and aerated with carbon-dioxide-free air and at 23° C.

In all the above cases the unspecified details conform to the requirements of the technique and apparatus previously described (**39**). The volume of the solution was two liters, the number of discs 60, and the duration of the experimental treatment 70 hours.

At the outset it will be clear that the comparison of the tissue from treatment no. 1 with the corresponding initial tissue, evaluates the effect on the various metabolites of those processes which are independent of

salt accumulation and which are determined mainly by the variables temperature, aeration, and time.

The tissues subjected to treatment no. 2 by comparison with that obtained from no. 1 tests the possibility that, even in the dilute solutions from which accumulation occurs, the ions of a readily accumulated salt cause specific effects on the metabolic processes.

Treatment no. 3 provides all the conditions necessary for optimum activity in the cells but a salt concentration which, though it would be conducive to the absorption of an increased *amount* of salt, would suppress the degree of *accumulation*. Specific effects of these readily absorbed ions on metabolism which are determined by the *quantity* of salt absorbed should be accentuated in treatment no. 3 compared to treatment no. 2.

Treatment no. 4 should be compared with treatment no. 2; the differences in the cultures are attributable solely to the oxygen concentration. At the lower oxygen tension it would be expected that any metabolic process with which the accumulation of salt is directly connected would be small.

Treatments nos. 5 and 6 evaluate the effect on metabolism of specific ions which have a special interest in the problem of salt uptake. Treatment no. 5 provides an anion (NO_3) which is absorbed in excess of even the readily absorbed cation (K); and treatment no. 6 provides by comparison with treatment no. 2 a case in which the uptake of even the readily accumulated anion (Br) is retarded by the cation calcium which is more slowly absorbed.

Results

Effect of salts and oxygen on the absorption of ions

Table I records the total absorption of anion and cation by 45 gm. (initial weight) of potato discs and shows that certain expectations were fulfilled.

The analyses were most conveniently made on the external solution. The bulk of this was rather large and therefore in calculating the total amounts absorbed error may be incurred. The absorption of the anions, however, was also obtained by analyses on the dried tissue. For the case of bromide (nos. 1, 2, 3, 4, 6) the agreement is so close that great confidence can be placed in the analyses on the external solution alone. The determination of the uptake of potassium from analyses on the tissue dried after blotting yielded figures of the same order as, but somewhat less than, those in table I which are preferred because the data obtained from the tissue are complicated by the effects of blotting the discs.

The potassium which may leave the tissue in the first few hours (**38**) after immersion was re-absorbed and at the end of the experiment the

TABLE I

EFFECT OF THE TREATMENT ON THE WATER AND IONIC RELATIONS OF TISSUE AND SOLUTION

Treatment		Total mg. equivalents absorbed by 45 gm. of original tissue			Total mg. equiv. HCO_3 in residual external solution	Final pH external solution	Per cent. gain of fresh weight
Number	Conditions	Anion data from external solution	Anion data from dried tissue	Cation data from external solution			
						pH	%
1	Dist. H_2O, air, 23° C.	....	....	0.02	0.20	5.6	7.27
2	0.005 equiv. KBr, air, 23° C.	1.3	1.12	1.40	0.36	5.9	8.09
3	0.05 equiv. KBr, air, 23° C.	2.4	2.34	5.00	0.28	5.9	4.09
4	0.005 equiv. KBr, air, 3.80 per cent. O_2, 23° C.	0.5	0.55	0.70	0.24	5.9	8.24
5	0.005 equiv. KNO_3, air, 23° C.	4.12*	1.66† } 3.31‡ }	1.60	2.56	6.8	10.50
6	0.005 equiv. $CaBr_2$, air, 23° C.	0.96	1.04	.....	1.04	6.8	6.46

* Loss of nitrate by external solution.
† Gain of organic nitrogen by tissue—not including NO_3 or NO_2.
‡ Gain of total nitrogen by tissue including NO_3 or NO_2.

tissue exposed to distilled water retained its initial salt content. At low oxygen concentration (solution in equilibrium with 3.80 per cent. O_2) the quantity of both bromide and potassium absorbed was much less than in similar solutions which were in equilibrium with air. A tenfold increment of salt concentration caused approximately a twofold increase in bromide absorption (**40,** p. 225). In the cultures of potassium bromide, bicarbonate did not occur in the final solution in amounts greatly in excess of the amount contained in distilled water in which tissue had respired for the same period. This, together with the fact that the final pH of the salt (nos. 2, 4) and distilled water (no. 1) cultures was approximately the same, is in accord with previous evidence that potassium and bromide are absorbed from *dilute* solution in equivalent amounts by potato discs. In the strong solution (no. 3) the cation (K) was absorbed in excess of the anion (Br) and from the work of STEWARD and HARRISON (**46**) two distinct mechanisms may be involved in the uptake of the cation. The excess absorption of cation over anion in the experiments referred to was due to an uptake of the former by a mechanism which is independent of vital processes (the so-called "induced absorption") and which, unlike the accumulation of potassium bromide ("primary absorption"), is not retarded in strong salt solutions. From a solution of calcium bromide (no. 6) a smaller bromide uptake occurred than from the corresponding potassium

salt (no. 2), the final solution became more alkaline and contained an amount of bicarbonate ion equivalent to the bromide removed. These facts indicate that much, if not all, of the bromide from calcium bromide was absorbed unaccompanied by cation and replaced in the external solution by bicarbonate ion (compare (**18**, p. 498)). Similarly, the nitrate ion was absorbed in excess of the cation (K) from a solution of potassium nitrate (no. 5) and the total uptake of anion was greater than that of bromide under otherwise similar conditions (no. 2).

The determination of the amount of nitrate absorbed by the cells is complicated by several factors. A *minimum* figure for nitrate absorption is given by the gain of total organic nitrogen by the tissue. This figure may be too low if nitrate remained unmetabolized in the cells as nitrate or nitrite. Both the gain of organic nitrogen and of total nitrogen including nitrate and nitrite were determined and, as shown by table I, even the latter figure (3.31 mg. equiv.) is less than the decrease of nitrate in the external solution (4.12 mg. equiv.). The latter figure may be in excess of the nitrate absorbed owing to reduction of nitrate by reducing substances potato tissue contains—substances[4] which were found in leached extracts of living tissue (**37**). In the light of the work of PEARSALL and BILLIMORIA (**28**) however, this discrepancy may be caused by decomposition of amino compounds and subsequent loss of nitrogen in molecular form in which case an equal amount ($4.12 - 3.31 = 0.81$ mg. equiv.) of amino nitrogen must also have disappeared from the tissue. In any event it is significant that the excess absorption of nitrate over potassium ($4.12 - 1.60 = 2.52$ mg. equiv.) is almost exactly balanced by the residual bicarbonate (2.56 mg. equiv.) in the external solution.

THE EFFECTS OF SALTS AND OXYGEN ON WATER ABSORPTION

There is an evident contrast between the effect on water absorption of treatments nos. 2 and 5 on the one hand and no. 6 on the other. In nos. 2 and 5, despite the presence of an external solute, the tissue gained *more* water than from water free from salt (no. 1) whereas treatment no. 6 produced an uptake of water significantly less than that of treatment no. 1. Reason may be sought in some specific effect of the ions and is not due to the osmotic value of the salts. Starch hydrolysis causes an increase of osmotic pressure in the sap but the final sugar concentrations (table III) which fall in the order treatment no. $5 < 2 < 1 < 6$, clearly cannot explain the water absorption figures which are in the order no. $5 > 2 > 1 > 6$. The same is true of the salt absorption data since by this criterion the calcium

[4] The reducing properties of these leached extracts are not attributable to their content of ascorbic acid, although they were first observed before the reactions of this substance became known.

culture again absorbs an unexpectedly small amount of water. The presence of potassium promotes water absorption in potato discs in aerated solutions whereas the presence of calcium depresses it and this is not due to *any known effect on the osmotic pressure of the cell sap.* The outstanding result of treatment no. 5 shows that when accompanied by nitrate the effects due to the potassium ion are accentuated. The same effect will later be evident on other aspects of the behavior of potato cells, *e.g.*, respiration and protein synthesis. These effects suggest that the water uptake of the cells is linked with the vital processes mentioned. Extreme modifications in the external and internal solute concentration do influence the water uptake as expected from simple osmotic theory. The comparison of treatments nos. 2 and 3 shows that after a tenfold increase in salt concentration the specific effect of the potassium salt is masked by the osmotic effect of the stronger salt solution. Similarly, at low oxygen concentration (treatment no. 4) though absorption is suppressed and the vital processes operate at a minimal rate the tissue still absorbed water; but this could be attributed to the fact that with reduced metabolism these cells retained a higher sugar concentration than other cultures (nos. 2 and 5) in potassium salts of the same concentration.

The water absorption of potato discs is not explained, therefore, wholly in terms of the osmotic value of the external solutions even the known factors (total salt and sugar concentration) which affect the osmotic pressure of the cell sap. Effects of salts on the permeability of the protoplast to water, of the kind which have been described by BAPTISTE (**2**) might affect the rate at which osmotic equilibrium is established but should not determine the total amount of water absorbed. Also, there is little experimental basis for the possible suggestion that the effects described could be due solely to effects on the properties of the cell wall.

The mechanism of water absorption involves a somewhat baffling array of variables many of which also affect metabolism. At the same equivalent salt *and oxygen* concentration, that treatment which caused the greatest protein synthesis (no. 5) produced the maximum water absorption *despite the fact* that it depleted the soluble reserves (sugar and amino-acids); whereas the treatment (no. 6) which, although it conserved the reserves of sugar yet depressed the synthesis of protein, also decreased the water absorption. Though the water absorption data here recorded are admittedly few they are supported by much other evidence and they refer to cells the metabolic history of which was extensively investigated. Therefore, despite the seeming over-emphasis, it is suggested that actively metabolizing cells which can grow, may absorb water in a manner which has but little relation to any conventional osmotic or suction pressure theory but may be more directly linked with metabolic processes (respiration and protein

synthesis); processes which are determined by oxygen and affected by the nature of the salts present in the external solution.[5] Relevant to this standpoint is the recent work of BENNET-CLARK *et al.* (**5**). These authors definitely contemplate the possibility that water absorption, like salt accumulation, is not merely an equilibrium process but may involve a mechanism of "internal secretion" which is peculiar to cells which are still able to grow. In this connection the brief statement of ERNST (**14**) that water may flow across a membrane against the hydrostatic gradient if there is maintained a temperature difference raises a possibility of the greatest interest.

Investigations of the water absorption of potato tissue have dealt with the effect of suction pressure (**50, 51**); the permeability, real or supposed, of the cells to water (**2, 49**); the use of water content as an index of toxicity [series of papers commencing with STILES (**48**)] or with the supposed connection between the colloidal swelling of ampholytes, particularly protein, and the water absorption of the cells (**26, 27, 35, 36**). Remarkably little attention has been paid to the variables which affect vital activity and metabolic processes, although an exaggerated emphasis has been placed upon certain small effects of external pH on water absorption, to the neglect of those variables (oxygen concentration, presence of neutral salts) which have little demonstrable effect on the swelling of colloids *in vitro* but do have a great effect upon water absorption and metabolic processes. That the colloidal properties of ampholytes may contribute to the water relations of cells can hardly be disputed but it seems equally evident that the rôle of the proteins and nitrogen metabolism in water uptake is a more intimate one in organized growing cells than the mere passive behavior which *in vitro* swelling experiments reveal.

The effects of nutrition on water content and nitrogen metabolism of growing plants emphasize that they are interrelated. This is shown by

[5] Since the above was written the paper of REINDERS (**34**) has appeared. REINDERS explores the possibility that water absorption, like salt absorption, is linked to vital activity—a standpoint for which there is much to be said. No conclusion is possible, however, from his data. It is true that REINDERS observes again, as one of us (F.C.S.) has often done in the past, that treatments which increase respiration in distilled water (and also salt uptake in salt solutions) also increase water absorption. The means by which REINDERS determined respiration (loss of dry weight) is however, excessively crude and, in the light of the various other processes in the tissue and involved in the technique of the experiments, is certainly misleading. As data on the actual concentrations of organic solutes (particularly sugar) in the experimental tissue is lacking (compare table III and fig. 2 of this paper) it cannot be concluded from REINDERS' paper that there is any discrepancy between the observed facts and the expectations of conventional osmotic theories of water absorption. It is of interest that dilute solutions of auxin influence the dry weight and the water uptake of potato discs; but this fact alone does not warrant the view that water uptake is anything more than a simple osmotic phenomenon.

the well-known relation between nitrogen supply and succulence, which was examined by PEARSALL and EWING (27). To cite but a few recent papers, those of GREGORY and SEN (18), confirming earlier results of RICHARDS (33), and PETRIE and WOOD (29) agree that nutritional treatments which during growth produce plants with a low net protein content also produce a low water content and, in a general way, the converse is true. PETRIE and WOOD discuss their data from the standpoint that water content determines the concentrations of nitrogenous constituents in the cells which in turn regulates the level of protein maintained; but in a final sentence they recognize the alternative view that "it is possible that the protein content is also a factor determining the water content to some extent." The results of tables I, II, and III suggest that aerobic respiration, protein synthesis, water absorption, and salt accumulation are all mutually dependent processes which occur in cells which are not subject to equilibrium conditions but the behavior of which, at constant temperature, is regulated by oxygen tension and the nature and concentration of the salt solution in which they are immersed.

EFFECT OF SALTS AND OXYGEN AT 23° C. ON RESPIRATION

Table II records the effect of the different treatments on respiration.

TABLE II

EFFECT OF SALTS AND OXYGEN UPON RESPIRATION

TREATMENT		RESPIRATION		TOTAL* RESPIRATION CO_2
		RATES MG. CO_2 PER GM. HOUR		
NUMBER	CONDITIONS	PERIOD 1	PERIOD 2	
		mg.	*mg.*	*mg.*
1	Dist. H_2O, air, 23° C.	0.204	0.220	683.0
2	0.005 equiv. KBr, air, 23° C.	0.180	0.237	706.0
3	0.05 equiv. KBr, air, 23° C.	0.222	0.289	867.0
4	0.005 equiv. KBr, 3.80 per cent. O_2, 23° C.	0.109	0.081	301.0
5	0.005 equiv. KNO_3, air, 23° C.	0.205	0.304	942.0
6	0.005 equiv. $CaBr_2$, air, 23° C.	0.178	0.188	640.0

* Corrected for bicarbonate content of the external solution.

The effect of oxygen, demonstrated elsewhere (40 and references cited in 42 and 43), is again shown by the comparison of treatments nos. 2 and 4. In the comparison of all other treatments (nos. 1, 2, 3, 5, 6) the considerable differences in respiration recorded (ranging from 640 to 942 mg.) were produced by the salt supplied. By oxygen concentration alone the respiration of potato tissue cannot be increased much above the value which it has

in distilled water in equilibrium with air although, owing to the external salt supply, increments of the order of 50 per cent. are here recorded at oxygen concentrations at which, on the former evidence, the respiration should be limited by factors other than oxygen. Clearly, therefore, the effect of salts on respiration is *through that factor, or group of factors, which in the earlier experiments was limiting at oxygen concentrations greater than those of solutions in equilibrium with air.*

The greatest response was obtained in dilute potassium nitrate and this suggests that the factor affected involves the nitrogen compounds. The outstanding effect of nitrate on respiration of potato discs, though somewhat unexpected in a tissue as rich in stored soluble nitrogen, is in agreement with many other recorded observations; an outstanding similar example being that recorded by HAMNER (**19**) in which large increments of respiration of high carbohydrate tomato plants occurred when nitrate was supplied. The comparison of treatments nos. 1, 2, and 3 indicates that as the total absorption of potassium bromide was increased there was a concomitant increase in carbon dioxide production. As in a previous paper (**47**) no such increase of respiration occurred in equivalent strengths (compare nos. 2 and 6) of calcium bromide; on the contrary, a significant decrease was observed. The cause of these salt effects on respiration must be sought in some factor which is affected reciprocally by the cations potassium and calcium and this is only apparent from the data on nitrogen metabolism (table IV and fig. 1).

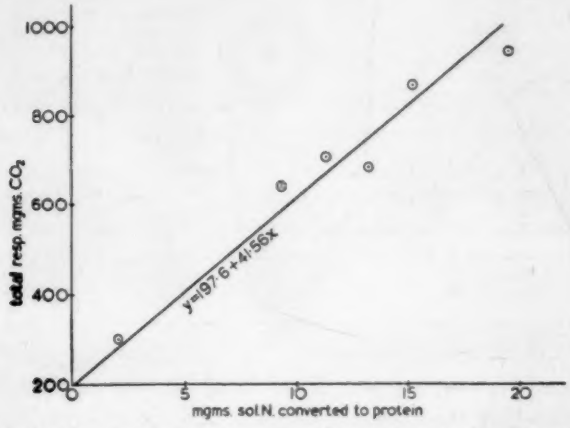


FIG. 1. Relationship between carbon dioxide respired and synthesis of protein in potato discs at 23° C.

It will be observed that the effects of salts on the respiration of potato tissue are distinct from, and opposed to, the view of LUNDEGÅRDH (**24**) who first ascribed all the effects of salts to the anions but who now admits that

there is a slight effect attributable to the cations although this appears only when there is intensive anion absorption (**23**). LUNDEGÅRDH further believes that the anions exert their effect through a special component of respiration which is a function of the anion actually absorbed. It will be noted that the cation effects are apparent in very dilute solutions and also that, in calcium bromide solutions (tables I, II) there was a considerable absorption of bromide which was accompanied by a *lower* respiration rate than that which obtained even in distilled water. The absorption data in table I and the total respiration figures in table II cannot be fitted into a scheme of the kind described by LUNDEGÅRDH or an equation of the form $R_t = R_g + KA$ where K is a constant specific for the anion and A the amount absorbed. This is apparent if the calculations are made from the data of tables I and II with reference to those treatments which involve only distilled water or bromides. Since the expression breaks down in its application to the bromide cultures *alone* there is no reason to discuss LUNDEGÅRDH's latest views (**23**) which concern the relationship between respiration as it occurs in salt solutions and in distilled water.

That salts and oxygen at 23° C. affect metabolic processes other than respiration is shown by their effect (tables III, IV, V) on the various metabolites of the discs. The analytical determinations, to which earlier reference has been made, were applied to the tissue and, where necessary, to the final external solutions. The data were calculated to the basis of 60 discs and 45 gm. of initial fresh weight (see also **47**).

EFFECTS OF SALTS AND OXYGEN AT 23° C. ON STARCH AND SUGAR CONTENT OF POTATO DISCS

The results of the carbohydrate determinations will be considered later in relation to other metabolites. First, certain general points need emphasis.

In all cases (see table III, treatments nos. 1 to 6) in which discs were exposed for 70 hours to the conditions used for the salt accumulation experiments they acquired sugar concentrations much in excess of their initial condition (table III, no. 7). The figures for starch determination show that very considerable quantities of starch disappeared—a fact which can be demonstrated microscopically (**53**).

Comparison of treatments nos. 2 and 4 shows that at the low oxygen concentration which retards the metabolism of the tissue, less hydrolysis of starch occurred. Potassium bromide cultures had no appreciable effect on the starch hydrolysis which was determined rather by time, oxygen, and temperature (compare nos. 1, 2, 3). Comparison of salt treatments nos. 2 and 6 shows that a calcium salt decreased starch hydrolysis.

The effects of the various treatments on the residual sugar concentration are clear. In cultures in equilibrium with air (nos. 1, 2, 3, 5, 6) the

TABLE III

EFFECT OF SALTS AND OXYGEN UPON STARCH AND SUGAR CONTENT DURING 70 HOURS AT 23° C.

CONDITIONS		STARCH CONTENT GRAMS PER 45 GRAMS OF ORIGINAL DISCS	SUGAR CONCENTRATION*	
NUMBER	TREATMENT		GRAMS PER 45 GRAMS ORIGINAL DISCS	GRAMS IN 2 LITERS OF EXTERNAL SOLUTION PER 45 GRAMS OF ORIGINAL DISCS
		gm.	*gm.*	*gm.*
1	Dist. H_2O, air	3.04	0.517	0.035
2	0.005 equiv. KBr, air	3.03	0.454	0.050
3	0.05 equiv. KBr, air	3.05	0.393	0.048
4	0.005 equiv. KBr, 3.80 per cent O_2	3.77	0.506	0.040
5	0.005 equiv. KNO_3, air	3.22	0.361	0.052
6	0.005 equiv. $CaBr_2$, air	3.51	0.568	0.056
7	Original washed discs	4.29	0.220	.....

* Calculated as glucose.

salt treatments which increase respiration (nos. 2, 3, 5) decrease the final sugar concentration. The increased sugar concentration which occurs at the cut potato surface has been regarded as the cause of the high respiration which this treatment produces (**22**). Clearly, under the above treatments, the *highest* respiration rates are associated with the *lowest* sugar concentrations (compare tables II and III). Thus sugar appears to be related to the respiration merely as the source from which carbon is drawn and not as the immediate respiratory substrate the *gross concentration* of which regulates the respiration rate.

It is possible, though perhaps improbable, that a detailed analysis of the individual sugars might reveal one more closely related to the respiration rate than the total sugar concentration. In the experiments of HOPKINS (*loc. cit.*) the proportion of reducing sugar to total sugar varied in different experiments from approximately 35 to 70 per cent. but within each experiment it remained constant and was subject only to casual variations. In other experiments[6] both the reducing sugar and disaccharide content of standard discs of potato after exposure to the same conditions of temperature and aeration as were used in the experiments of table III has been determined at various times during storage. The ratio hexose: sucrose varied from 0.98 to 1.4 with a mean at 1.14. Of the final sugar residue approximately half is hexose.

The relation of the respiration of whole tubers to their sugar content has been exhaustively investigated by BARKER (**3, 4**) who found that when high sugar content was produced by low temperatures this also caused the

[6] Unpublished data. Botany Dept., Birkbeck College.

accumulation of a respiratory inhibitor. The mutual inter-action of sugar concentration and inhibitor on the respiration rate is complex, but BARKER concludes that it is the sucrose concentration, not the total sugar concentration, which is most closely correlated with the respiration rate of whole tubers. In the treatments studied by DENNY *et al.* (11) the high respiration precedes the attainment of high sugar concentrations and these workers believe that organic acids (citric) are the immediate respiratory substrates. The point of view of this series of papers is, however, that the effects induced by salts on the respiration rate of potato discs are not attributable to their effect upon the concentration of sugars but to factors which become apparent only when the nitrogen metabolism is appreciated.

EFFECT OF SALTS AND OXYGEN ON NITROGEN METABOLISM AT 23° C.

The effect of the different treatments previously described on the alcohol soluble and alcohol insoluble nitrogen fractions is shown in table IV.

TABLE IV

EFFECT OF SALTS AND OXYGEN ON NITROGEN METABOLISM OF POTATO DISCS DURING 70 HOURS AT 23° C.

TREATMENT		SOLUBLE N AS PER CENT. OF TOTAL RECOVERED N	PROTEIN N AS PER CENT. OF TOTAL RECOVERED N
NUMBER	CONDITIONS		
		%	%
1	Dist. H_2O, air, 23° C.	50.0	50.0
2	0.005 equiv. KBr, air, 23° C.	51.9	48.1
3	0.05 equiv. KBr, air, 23° C.	47.6	52.4
4	0.005 equiv. KBr, 3.80 per cent. O_2, 23° C.	61.4	38.6
5	0.005 equiv. KNO_3, air, 23° C.	67.2*	56.4*
6	0.005 equiv. $CaBr_2$, air, 23° C.	54.0	46.0
7	Original washed discs	63.5	36.5

* Expressed as percentage of initial total N.

Analysis of the external solution showed that the total amount of nitrogen in the two liters of solution was negligible. Hence the nitrogen effects can be dealt with by reference only to the tissue. The sum of the alcohol insoluble nitrogen fractions calculated to the basis of 45 gm. of initial tissue (60 discs) gives an estimate of their total nitrogen content. The combined variation in total nitrogen is due to inherent variability of the tissue and the errors of determining the nitrogen fractions. From seven determinations the mean total nitrogen content of 60 discs (45 gm. initial weight) was 98.6 mg. and apart from treatment no. 5, in which the tissue gained in total nitrogen by 23.6 per cent., the discrepancy between the total nitrogen recovered and the mean total nitrogen content never exceeded 2.5 per cent.

Treatment no. 1 shows that during 70 hours at 23° C. in aerated distilled water, the tissue synthesized protein (13.3 mg. of protein nitrogen per

45 gm. of initial discs) at the expense of the soluble nitrogen reserves. STUART and APPLEMAN (*loc. cit.*) have also recorded an increase, although very slight, of protein content in potato slices, which presumably had a very small specific surface, during wound periderm formation. As in our experience, the synthesis occurred at the expense of α-amino-nitrogen reserves. The synthesis of protein from stored soluble nitrogen compounds is clearly dependent upon oxygen (compare nos. 1 and 4); at the low partial pressure of 3.80 per cent. (no. 4) the loss of soluble and the gain of protein nitrogen was hardly significant.

The effects of salts, which are superimposed upon the effects of oxygen, are evident from the comparison of treatments nos. 1, 2, 3, 5, 6.

As expected, the nitrate treatment (no. 5) caused a gain of total nitrogen, of soluble nitrogen, and also a greater protein synthesis than in any other culture.

The calcium salt (no. 6) which depressed the respiration, carbohydrate metabolism, and water absorption, also retarded protein synthesis,[7] whereas the stronger potassium salt,[8] which increased respiration and the utilization of carbohydrate, also caused an increase in the synthesis of protein at the expense of the soluble nitrogen fraction.[9]

RELATION BETWEEN THE EFFECTS OF SALTS AND OXYGEN UPON RESPIRATION AND PROTEIN SYNTHESIS AT 23° C.

There is an evident parallelism between the effects on respiration and protein synthesis of the treatments investigated. Low respiration is associated with low protein synthesis (no. 4) and the highest respiration with the greatest protein synthesis (no. 5). Figure 1 shows graphically the approximately linear relationship between the total respiration–y (mg. of CO_2 respired, for data see table II), and the mg. of soluble nitrogen converted to protein–x, [gain of protein N (table IV) as percentage of total N $\times$ 98.6 mg.]. The line drawn represents the closest fit and has the equation $y = 197.6 + 41.56x$. The conclusion follows that for this range of treatments, per mg. of nitrogen in the protein synthesized, the associated increase of respiration is equal to 41.56 mg. of carbon dioxide or, per equivalent of nitrogen converted to protein, an additional 13.2 mols of carbon dioxide

[7] The differences between treatments 1 and 6 are not large, but they are confirmed by a large amount of evidence which will be presented in a subsequent paper.

[8] The small differences between treatments 1 and 2 cannot be regarded as real since they have not been confirmed in the extensive experiments to be discussed later.

[9] These effects of cations should be clearly distinguished from the effects of acute mineral deficiency. In the cells concerned neither potassium nor calcium were at this low level. The fact that a calcium salt decreases protein synthesis in potato discs does not indicate that calcium is unnecessary for this process; when calcium is supplied at concentrations which limit growth, protein synthesis may be limited by the lack of calcium.

were formed over and above a basal carbon dioxide production which occurred independently of protein synthesis. From an arbitrarily selected range of conditions the arithmetical relationship between one equivalent of soluble nitrogen synthesized to protein and two (strictly 2.2) molecules of hexose aerobically oxidized to sugar should not be overemphasized. The important point is that the effects of salts and those of oxygen upon respiration are exerted through the same component of the total respiration and this component, moreover, is closely linked with protein synthesis.

The explanation of the connection between respiration and nitrogen metabolism is not immediately apparent from these results. A full discussion would entail a more extensive treatment of the literature of nitrogen metabolism, especially as it is affected by nutrition, carbohydrate supply, and oxygen, than can be included in this paper. Although it will be amplified in subsequent papers, the approach to the problem can be stated briefly for present purposes as follows.

The soluble nitrogen of potato discs consists of amino acids and amides (**47**). Usually amino acid predominates over amide and as shown previously it is from this fraction that the nitrogen for synthesis is derived. It is postulated that the amino acid liberates ammonia by oxidative deamination and that this process links protein synthesis, respiration, and the effect of salts and oxygen. As shown by RAPER and his colleagues (**32**), the effective oxidizing agent of the aerobic oxidase system of the potato is an ortho-quinone derived from catechol or a substance which contains this grouping and, from the work of HAPPOLD and RAPER (**20**), it is also known that this oxidizing agent deaminates amino acids (*e.g.*, glycine, alanine, phenylalanine). More recently DAMODARAN and NAIR (**9**) have described a specific aerobic dehydrogenase which converts glutamic acid to α-ketoglutaric acid; this enzyme has been demonstrated only in seeds. In a previous paper (**47**), it was shown that the treatments with inorganic salts and oxygen which affected respiration and protein synthesis similarly affected in a visible manner the activity of this oxidizing system within the living cells of potato discs. It follows from the large amount of carbon dioxide which accompanied the metabolism of each equivalent of nitrogen (fig. 1) that the carbon residue from deamination of the amino acids supplied a small part of the total carbon involved in respiration; and the balance, as also the carbon necessary for protein synthesis, must have been derived from sugar or organic acids or both. The large consumption of carbohydrate shown in table III offers ample evidence, if such were needed, of the metabolism of carbohydrate. Reference to the earlier paper (**47**), however, shows that organic acids are similarly consumed and in the balance sheet of carbohydrates (**42**), the carbohydrate used was accounted for without assuming that it was converted to protein.

Hence sugar and amino acids bear to respiration and protein synthesis a similar relationship. They represent the sources from which the nitrogen and carbon are drawn, their *individual concentrations do not regulate respiration* but their utilization is dependent upon the activity of the same oxidizing system which is in turn regulated by the presence of salts and oxygen. This somewhat unconventional point of view finds its closest parallel in the interesting account given by GREGORY and SEN of the respiration of barley leaves subjected to different levels of mineral nutrition (**18**). A close comparison of the case of barley leaves and potato discs and a more detailed discussion of the relation between nitrogen metabolism and respiration will be deferred until more effects of salts on these processes have been presented in a later paper and until the effects of the ions on the components of the soluble nitrogen have been investigated. The oft cited work of SPOEHR and MCGEE focussed attention on the effect of amino acids on respiration. Since then the relation between nitrogen metabolism and other physiological functions, especially photosynthesis and respiration, and the way this is modified by other inorganic nutrients (*e.g.*, K) has become a major problem [see reviews of mineral nutrition of plants in ANNUAL REVIEWS OF BIOCHEMISTRY and a recent review by NIGHTINGALE (**35**)]. Our purpose here is to establish the connection between nitrogen metabolism and respiration for potato discs, to emphasize that it is the processes of synthesis from amino acids which are concerned in this relationship, and to postpone the fuller discussion of this problem and the relevant literature which is necessary.

THE COMPONENT OF RESPIRATION NOT LINKED TO PROTEIN SYNTHESIS

There is, however, a large part of the total respiration of potato discs at 23° C. which does not appear to be linked with protein metabolism and this can be estimated from the equation of figure 1 as the value of the total respiration ($y = 197.6$ mg. CO_2) at zero protein synthesis ($x = 0$). Expressed as an average rate this yields the figure 0.062 mg. CO_2 per gm. initial fresh weight per hour which can represent only the value of that component of the total carbon dioxide production which is not affected by the salt and oxygen treatment, is not related to the metabolism of the nitrogen compounds, and which earlier papers have shown is not concerned in salt accumulation (**44**). An independent estimate of the value of this component of respiration may be made from the effect of oxygen tension upon the respiration of similar potato discs at 23° C. which respired for the same total time period (70 hours) in distilled water or salt solutions so dilute that the specific effect of the salt can be neglected (**40**). If the respiration of potato discs may be partitioned into two components, the one linked to protein synthesis and the other independent of it, then the former should

vanish at zero oxygen tension and the probable value of the latter may thus be derived from the value which the respiration approaches at zero oxygen pressure (see (40), fig. 2). Hence the component independent of nitrogen

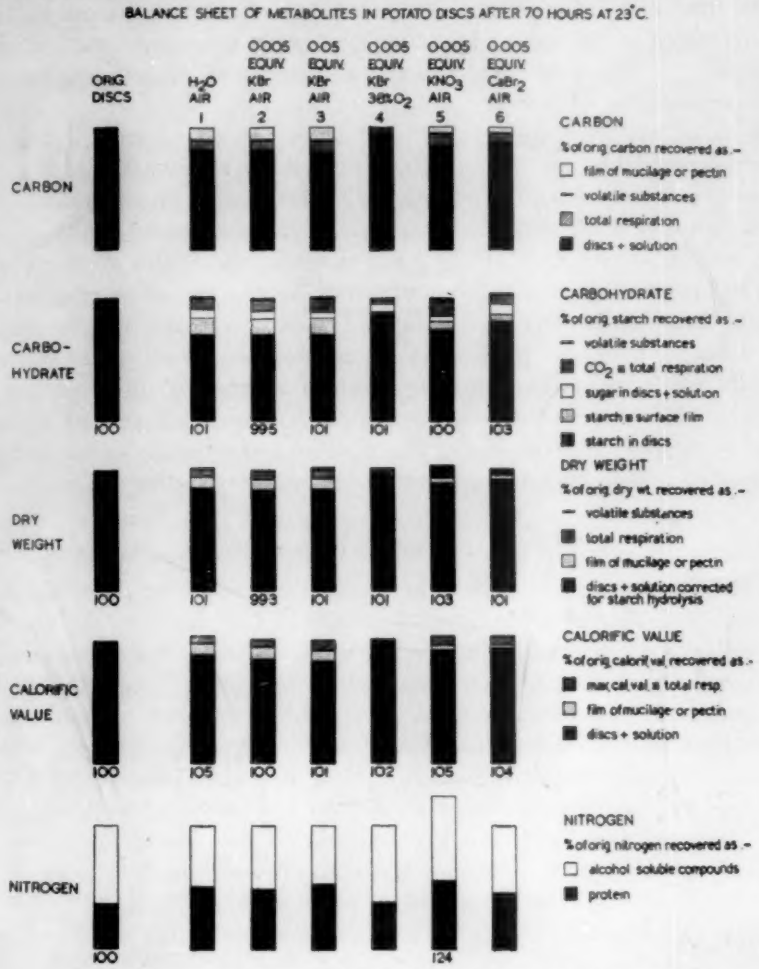


FIG. 2. Balance sheet of metabolites in potato discs at 23° C.

metabolism is estimated at 30 per cent. of the respiration of discs in dilute solutions in equilibrium with air at 23° C. and, for the case in question, would correspond to a rate of 0.072 mg. CO_2 per gm. per hour, a figure which agrees closely with that of 0.062 mg. per gm. per hour obtained above. This agreement can hardly be fortuitous and must surely mean that approximately one-third of the respiration of standard potato discs at 23° C. in distilled water or dilute salt solution is attributable to a respiratory

component which is not directly affected by variations in *oxygen concentration*, is not related to the nitrogen metabolism, or affected by the presence of salts and is not a factor in salt accumulation. The remaining two-thirds of the total respiration of the discs at 23° C. clearly represents that component which is influenced by salts, by oxygen concentration, and which is intimately concerned both with the mechanism of protein synthesis and salt accumulation of potato discs.

It is of the greatest interest that since the above was first written BOSWELL *and* WHITING (**8**) *have investigated the relationship of the respiration of potato discs to the polyphenol oxidase which they contain. Their conclusion is that the respiration consists of two components, both of which have an R.Q. = 1.0. One of these components contributes 66 per cent. of the total respiration and is brought about by the oxidation of a phenolic body to its oxidized form by oxidase and subsequent reduction, by a hydrogen donator, with the production of compounds which split off carbon dioxide. The identity of this respiratory component of* BOSWELL *and* WHITING *with that described above which is linked with protein synthesis seems hardly to be questioned. The oxidase and phenolic substances play an essential role in both and contribute the same proportion* ($\frac{2}{3}$) *of the total respiration.*

THE BALANCE SHEET OF METABOLISM

Thus far the way in which the metabolism of potato discs is affected by salts and aeration has been described. The recovery of the total metabolites may be tested in several ways. Each culture contained 60 discs from which duplicate and random samples of 30 discs were obtained and used for the determinations of dry-weight, sugar, starch content, calorific value, and carbon content. These determinations were also repeated on "initial discs" and all analytical data calculated on the basis of 45 gm. of initial fresh weight of washed discs. Since the total respiration was known and the small amount of organic matter present in the external solution was measured, a balance sheet may be prepared with respect to dry weight, carbohydrate calculated in terms of starch, calorific value, and carbon. This method enables the absolute recovery of the various metabolites to be measured.

Since the complete analytical technique was applied to two batches (A and B) of 30 discs random sampled from the 60 discs of each culture, all the determinations were carried out at least in duplicate. Where great certainty was desired replicate determinations were made on each of the samples A and B. From the values recorded for samples A and B the mean value for the tissue in each culture was obtained and this, when converted to the initial fresh weight basis, was the figure used in the balance sheet calculations. The duplicate tissue samples permit the effect of the

very small variability of potato tissue to be assessed. The full data will not be given here but it may be stated that the errors incurred by the variability of the material are small compared to the differences to which significance is attached (see also **47**).

To the observed final dry weight of the tissue was added a small correction (of the order of 0.040 gm.) which represents the dry weight of the organic matter in two liters of external solution and, unless the metabolite in question was directly determined (*e.g.*, sugar, total dry matter), it was assumed that this had the same percentage composition (heat and carbon content) as the dry material of the tissue sample. The estimate of organic dry matter in the external solution was obtained by the loss of dry weight[10] when an aliquot part was ignited and, especially in cultures nos. 3 and 6, it was necessary to correct this figure for the loss of weight sustained by a parallel sample of the same salt content, but free of organic matter, which received identical treatment. Under the conditions of drying, calcium bromide is hydrated and after ignition is anhydrous, whereas potassium bromide loses appreciable weight on ignition owing to the volatility of this salt. The corrections applied compensate for these sources of error.

Certain assumptions must be made in the balance sheet calculations. The expected effect of respiration on the total dry weight, carbohydrate, and heat content of the tissue can be estimated only in accordance with the familiar equation:

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O + 674{,}000\ \text{cal.}$$

despite the emphasis in an earlier section on the linkage between the respiratory processes and nitrogen metabolism which this equation does not reveal. The balance sheet method therefore tests whether the relationships between respiration and nitrogen metabolism involve drastic departures from this fundamental equation or only concern the intermediate steps by which the process is achieved. It has been assumed that the direct effect on dry weight, heat content, and carbon content of protein synthesis from soluble nitrogen compounds is negligible. Also, the effect of the reactions which involve organic acids on these properties was not evaluated. The significant effect on the dry weight caused by the hydrolysis of starch to sugar can be calculated from the observed gain in sugar content. The actual dry weight of the salt absorbed is small and has been neglected for present purposes. Within these limitations the results of table V lead to the following conclusions.

A complete account (accurate to 1 per cent.) of the fate of the dry weight, starch, heat, and carbon content of potato discs during their respi-

[10] For an estimate of the carbon present in the external solution reference may be made to later experiments (table VIII). For the proportions of tissue to solution in the above experiment this figure corresponds to 0.010 gm. of carbon in the external solution.

TABLE V

RECOVERY OF DRY WEIGHT, CARBOHYDRATE, AND CALORIFIC VALUE IN POTATO DISCS AFTER 70 HOURS OF METABOLISM AT 23° C. ALL QUANTITIES EXPRESSED PER 45 GRAMS (60 DISCS) OF INITIAL TISSUE

TREATMENT		DRY WEIGHT OF DISCS*	DRY WEIGHT LOSS BY RESPIRATION	GAIN OF DRY WEIGHT BY STARCH HYDROLYSIS†	STARCH CONTENT	STARCH USED IN RESPIRATION	STARCH EQUIVALENT TO GAIN OF SUGAR‡	HEAT CONTENT‡	HEAT EQUIVALENT OF RESPIRATION	CARBON CONTENT‡	CARBON EQUIVALENT TO CO_2 RESPIRED	PERCENTAGE RECOVERY OF INITIAL			
NUMBER	CONDITIONS											DRY WEIGHT§	STARCH	HEAT	CARBON
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*kgm. cal.*	*kgm. cal.*	*gm.*	*gm.*	%	%	%	%
1	Dist. H_2O, air	5.52	0.413	0.033	3.045	0.417	0.300	22.88	1.73	2.080†	0.185	92.3	87.9	95.6	89.6
2	0.005 equiv. KBr, air	5.40	0.421	0.028	3.026	0.421	0.257	21.95	1.75	2.073	0.193	90.6	86.5	91.9	89.5
3	0.05 equiv. KBr, air	5.42	0.512	0.022	3.050	0.513	0.200	21.53	2.13	2.033	0.228	92.4	87.8	91.8	89.5
4	0.005 equiv. KBr, 3.80 per cent. O_2	6.26	0.190	0.033	3.775	0.186	0.294	25.26	0.77	2.415	0.083	100	99.5	101	99.0
5	0.005 equiv. KNO_3, air	5.74	0.575	0.019	3.218	0.575	0.174	23.81	2.02	2.124	0.256	98.1	92.7	100	94.2
6	0.005 equiv. $CaBr_2$, air	5.96	0.384	0.040	3.506	0.384	0.364	24.34	1.60	2.235	0.170	99.0	99.4	100.5	95.0
7	Initial tissue	6.40	.....	.....	4.286	.....	.....	25.80	.....	2.528	.....	.....	.....	.....	.....

* Calculated from sample "B" only.

† Calculated from gain of total sugar in final tissue over original discs. To correct for probable proportion of disaccharide to total sugar (50 per cent.) reduce these figures by 25 per cent.

‡ Values corrected for the organic matter contained in the external solution. Sugar content of external solution by direct determination (table III). Dry weight of organic matter in external solution by loss of weight on ignition minus the change in weight of a parallel sample which contained the same amount of the principal salt.

§ Dry weight changes which are the direct result of salt absorption are here neglected. These are small and lacking complete data, for all the inorganic ions, are somewhat difficult to evaluate. The maximum correction could be calculated assuming equivalent uptake of anion and cation from the figures of table I but this is obviously too large because some salts of calcium and magnesium do leave the tissue in the early stages of these experiments (38) and are not reabsorbed completely. Protein synthesis cannot affect the dry weight of balance appreciably. The total dry weight of protein synthesis used in no. 1 amounted to little more than 5 per cent. of the total initial dry weight and clearly the change in dry weight during synthesis would be only a small fraction of this.

ration and metabolism at a low oxygen concentration (no. 4) and in contact with potassium bromide was obtained by the methods described. Similarly when respiration occurs in solutions of calcium bromide in equilibrium with air, the balance sheet shows a satisfactory account of the principal metabolites. This is not so, however, in the case of treatments 1, 2, and 3 for there are evident discrepancies in the balance sheet with respect to dry weight, starch, heat, and carbon recovery which far exceed the legitimate errors. These discrepancies cannot be attributed to any of the metabolic processes as yet described and they clearly indicate that a considerable portion of the stored starch of discs which are exposed to aerated water or potassium bromide is converted to a form such that under the conditions of the storage tissue experiments the *bulk* of the heat, dry weight, and carbon it contained is *lost entirely by the tissue system* (see table VI).

TABLE VI

DISCREPANCIES IN THE BALANCE SHEET FOR STARCH, DRY WEIGHT, AND CARBON

NUMBER	INITIAL DRY WEIGHT NOT RECOVERED	INITIAL STARCH NOT RECOVERED	INITIAL CARBON NOT RECOVERED	STARCH $(C_6H_{10}O_5)_n$ EQUIVALENT TO CARBON LOST
	gm.	*gm.*	*gm.*	*gm.*
1	0.493	0.518	0.263	0.593
2	0.601	0.578	0.266	0.600
3	0.486	0.523	0.266	0.600

Confidence in this unexpected result is not merely based on the agreement between the independent results for dry weight, starch, heat, and carbon content in tables V and VI but it has been confirmed in several similar experiments. From the data obtained on the parallel samples, A and B, of each culture and by the method of the "t" test (15) the probability that the observed difference between the mean values for initial and final discs could have been due to chance may be estimated. The data for calorific value yielded the figures in table VII and these show that the discrepancies in the "balance sheet" with respect to calorific value are real, a conclusion

TABLE VII

CALORIFIC VALUE. SIGNIFICANCE OF THE DISCREPANCY IN THE "BALANCE SHEET"

TREATMENT		t	P
NUMBER	CONDITIONS		
1	Dist. H_2O, air	18.4	< 0.01
2	0.005 equiv. KBr, air	22.0	< 0.01
3	0.05 equiv. KBr, air	11.0	< 0.01

which also applies to the similar discrepancies in the recovery of starch, dry weight, and carbon.

To sum up, therefore, there still remains an outstanding aspect of the metabolism of potato discs which occurs under the experimental conditions conducive to high respiration and salt absorption and which has not yet been evaluated. This metabolic process is one which causes, under the conditions of these experiments, almost the entire disappearance of the heat, dry weight, and carbon content of the carbohydrate involved. Apparently the reaction concerned demands a high oxygen tension, is suppressed in calcium salts, and in some degree by the nitrate ion (table V).

Fate of the unrecovered dry matter

It may be stated now that a detailed investigation of the volatile substances produced during the metabolism of potato discs revealed amounts of only a much lower order of magnitude than the outstanding losses of carbon evident in table VI.

Investigation showed that the bulk of the organic substance which was missing (table V) was removed from the surface of the discs on the filter paper used for the removal of surface water. Incidental to the technique of the salt accumulation experiments, discs in distilled water or potassium salts are slightly mucilaginous at the surface *before* they are blotted but this disappears when they are surface dried. This effect is somewhat more pronounced in the case of discs exposed to potassium salts than in those from distilled water cultures but is not observed in the *initial discs* which were cut and washed in running tap water.

Experiment showed the amount of carbon which may be removed from the surface of discs by drying paper and also that, if this loss of carbon is appreciated, a satisfactory account of the total carbon content of discs respiring in distilled water may be rendered.

Two parallel cultures (nos. 1 and 2, table VIII) of 40 discs were exposed to aerated distilled water at 23° C. for 90 hours. Each culture was then random sampled into two batches of 20 discs one of which was surface dried; the other was merely rinsed. Subsequently all the samples were vacuum dried and their dry weight and total carbon content (wet combustion) determined. On an aliquot part of the external solution the amount of carbon it contained was determined and the initial carbon content (812 mg.) was obtained on four replicate batches of twenty discs random sampled from the same stock. The total respiration during the 90 hours of the experiment was measured in the usual way.

Table VIII shows that the discs which were surface dried had lower dry weight and carbon content than the corresponding sample which was not blotted and that these differences are far greater than the differences

TABLE VIII

EFFECT OF BLOTTING THE DISCS ON THE RECOVERY OF TOTAL CARBON

Treatment			Dry weight per 20 discs	Carbon per 20 discs	Carbon respired per 20 discs	Carbon in external solution	Total carbon recovered per 20 discs	Percentage recovery of initial carbon	
Number	Conditions	Tissue							
			gm.	*mg.*	*mg.*	*mg.*	*mg.*	%	
1	Dist. H_2O, 23° C.	Blotted	1.752	674	82.5	3.3	760	93.6	92.9
2	" " "	"	1.687	662	83.6	3.3	749	92.2	
1	" " "	Unblotted	1.971	719	82.5	3.3	805	99.2	98.6
2	" " "	"	2.078	708	83.6	3.3	795	98.0	

between duplicate cultures. Table VIII also shows that whereas in the blotted discs there was a 7 per cent. discrepancy between the initial and final recovered carbon (which has a significance of $P = 0.01$) in the carbon "balance sheet" this was reduced to 1.4 per cent. (which by the "t" test (**18**) is not significant since $P = 0.4$) when the surface drying on filter paper was omitted.

NATURE OF THE SUBSTANCES REMOVED BY BLOTTING THE DISCS

From the data of tables V, VI, and VIII it is clear that the substance which was removed on the filter paper was produced at the expense of the starch fraction and contained the entire carbon content and at least 75 per cent. of the calorific value of the starch from which it was formed. Furthermore, it must have been a substance capable of forming a superficial, mucilaginous film on the external surface of the discs. The complete recovery of carbon compounds obtained in table V in the low oxygen culture (no. 4), the better recovery (no. 6) obtained if the tissue was exposed to solutions containing calcium rather than potassium even though these were in equilibrium with air, (as well as the complete recovery of total nitrogen which has repeatedly been obtained, even where the losses of dry weight occurred) all show that the substances in question *could not have been removed from within the cells by the slight pressure necessary to remove superficial water.*

The formation of a surface deposit on potato slices exposed to moist air is a familiar fact. The cut surface becomes blocked with a mucilaginous substance and the superficial cells quickly suberise (**30**). Although it was possible that the fatty substances which are concerned in suberization contributed to the surface film of carbon compounds which adhere to the filter paper, this was dismissed when a direct comparison of the fat content of blotted and unblotted discs was made. The probability that the substance removed was a pectin or mucilage directed attention to the uronic acid content of potato discs and the effect upon it of surface drying by filter paper.

As in the experiment of table VIII, discs were exposed to 2 liters of aerated distilled water at 23° C. for 67 hours in two cultures (nos. 1 and 2) each of 40 discs. The final tissue from each culture was sub-divided into two random samples (20 discs) one of which was blotted dry, the other merely rinsed, before being vacuum dried. On the dried ground sample the uronic acid content was determined by the method of DORE and the corresponding pectin content calculated. Table IX shows that a significant decrease in the uronic acid content of the blotted discs occurred and also that, on the basis of the somewhat arbitrary composition assigned to the pectin "molecule," such a substance could account for at least 57 per cent.

TABLE IX

REMOVAL OF DRY WEIGHT AND URONIC ACID DERIVATIVES FROM POTATO DISCS AFTER EXPOSURE TO AERATED SOLUTION AT 23° C.

Treatment			Pectin* per 20 discs	Pectin* removed by blotting	Dry weight removed by blotting	Carbon removed by blotting 20 discs	
Number	Experimental conditions	Tissue				Observed by total carbon determination	Pectin removed
			mg.	*mg.*	*mg.*	*mg.*	*mg.*
1	Dist. H_2O, air	Blotted	48.9				
2	" " "	"	49.3				
				174	305	50	39
1	" " "	Unblotted	226.0				
2	" " "	"	221.0				

* Calculated from decrease in uronic acid as a tetramethylated-tetragalacturonic acid-galactose-arabinose compound.

of the actual dry weight which was removed by blotting the discs and as much as 80 per cent. of the carbon removed. If, as is often the case, the particular pectin involved had a lower uronic acid content, or was associated with appreciable quantities of inorganic ions (*e.g.*, calcium and magnesium), the weight of dry matter per unit of uronic acid might be considerably greater. In fact, in earlier experiments it was noted that aerated potato discs in distilled water or potassium bromide solutions did lose surprisingly large amounts of magnesium which it was presumed accompanied organic anions into the external solution (**38**). In the light of this later evidence it is probable that much of the magnesium lost by the tissue accumulated with pectin or mucilage as a film at the surface of the discs and it adhered to filter paper when the discs were surface dried; in fact, the amounts of magnesium lost in the earlier experiments were of the right order assuming that each equivalent of uronic acid removed by the blotting technique (table IX) was associated with an equivalent of magnesium. The accumulated evidence is strongly in favor of the view that much of the magnesium lost by the tissue in these earlier experiments disappeared combined with uronic acids in a pectin-like complex.

It is clear, therefore, that during the metabolism of potato discs at 23° C. in aerated solutions some substance, which in the initial tissue was determined as starch, was converted to pectin-like substances which *accumulated as a film at the surface of the discs.* In running tap water or low oxygen cultures at 23° C. this did not occur. In potassium bromide solutions, or distilled water, the film formed was relatively fluid and adhered to filter paper, whereas in contact with calcium salts, if the film

formed at all, it remained precipitated on the discs in a form not removed by contact with paper. In the conversion of carbohydrate to uronic acid, oxidation is involved and it is noteworthy that treatment no. 4 of table V shows that at low oxygen tension (3.80 per cent O_2) even in the presence of potassium bromide solution the losses now attributed to the pectin-like substances did not occur. If the salivary extract used for starch hydrolysis also hydrolyzed the pectin-like compound[11] the data would not demand an actual synthesis of the complex compounds of uronic acid from starch but merely that at 23° C., in the *presence of oxygen* and the relative absence of calcium, pectin compounds already present became mobile and accumulated at the surface of the discs as a film. There is no obvious explanation of the apparent effect of nitrate which tended to depress the loss of pectins except that this ion stimulated carbohydrate metabolism and in consequence reduced the residual sugar concentration available for other reactions.

With this unexpected aspect of the metabolism of immersed potato discs the general picture of the metabolic processes which involve carbohydrate is almost complete. The only fractions not yet measured quantitatively are the volatile ones and these, which are produced in amounts too small to affect the "balance sheet" greatly, will be discussed in the next section.

Volatile carbon compounds produced by the potato discs at 23° C.

An intensive investigation of the volatile substances produced by potato discs under the conditions conducive to salt accumulation was undertaken by C. Preston, but this can be only briefly summarized.

During the salt accumulation experiments volatile substances could be lost by the system at three stages in the technique, namely: (a), with the respired carbon dioxide in the air stream used for aeration; (b), with the water evolved during the vacuum drying of the tissue; and (c), during the evaporation of the external solution.

The carbon compounds evolved along with the carbon dioxide were converted to carbon dioxide by passage through a three-foot combustion furnace packed as in standard combustion practice. During 70 hours the respiration rate of the tissue in dilute salt solution remained very constant (see (**47**) for form of time curve). Comparisons were made between the total carbon dioxide (CO_2 of respiration + CO_2 from volatiles) from one culture and the respired carbon dioxide alone from another. Similarly on the same culture the respiration rate alone, during periods when the volatiles were not burned, was compared with the total yield of carbon dioxide during periods when the volatiles were passed through the hot furnace. Elaborate blank experiments evaluated the traces of carbon dioxide obtained from volatile compounds which were not evolved by the tissue.

[11] See footnote 12.

The mean rate of total carbon dioxide production (0.205 mg. per gm. per hour) was greater when the volatiles were burned than in a parallel culture in which this was not done (0.189 mg. per gm. per hour). It was also greater than the mean rate (0.195 mg. per gm. per hour) of carbon dioxide produced by the same culture in periods when the furnace was cold. Differences between the respiration rate alone of parallel cultures or differences between the mean rates of the control culture from two series of alternating periods were not significant when tested by the "t" test. The extra carbon dioxide due to the combustion of the volatile substances (0.010 mg. per gm. per hour) on the contrary represents a real difference ($S = 4.9$, $n = 4$, $t = 2.5$: P somewhat > 0.05) especially since the only point at issue is whether the combustion technique caused a significant *increase* of carbon dioxide and, therefore, the value of P could be legitimately halved ($P = 0.025$). Therefore, the loss of carbon by volatile substances swept away with the air stream is equivalent to only 0.010 mg. CO_2 per gm. per hour, or approximately 5 per cent. of the respiration rate.

Investigation with carefully controlled blank experiments showed that no significant amount of combustible carbon compounds was evolved during evaporation of the external solution in which potato discs had been placed at 23° C. During 120 hours at 23° C. in aerated solution 115 gm. of standard discs accumulated volatile substance equal to 18.2 mg. of carbon which was evolved during vacuum drying. Hence the volatile substance accumulated in the tissue and the solution during 120 hours of metabolism at 23° C. did not exceed the equivalent of 0.0048 mg. of CO_2 per gm. per hour.

The maximum amount, therefore, of volatile compounds formed by potato discs at 23° C. in aerated salt solutions can be assessed as follows:

CO_2 equivalent to combustible compounds in air stream	= 0.010	mg. per gm. per hour	
" " " residual volatile compounds in tissue and external solution	= 0.0048	"	"
" " " total volatile compounds produced	= 0.015	"	"

The carbon dioxide equivalent of the total production of volatile compounds by standard potato discs at 23° C. is, therefore, approximately equal to 7.5 per cent. of the carbon dioxide evolved. An allowance of this amount for "volatiles" would increase the percentage of the total carbon accounted for in the case of unblotted tissue (table VIII) from 98.6 to 99.4 per cent.

Discussion

The corrected balance sheet of metabolites

A more complete balance sheet of the metabolites of potato discs showing the effect of the various treatments previously described may now be

prepared. The recovery of the initial carbon obtained in the discs plus solution and as respired carbon dioxide has been shown in table V. It has been proved that volatile carbon compounds accounted for only a very small amount of the unrecovered carbon (7.5 per cent. of the carbon evolved as carbon dioxide) and also that the carbon still unaccounted for was removed from the surface of the discs as a film of mucilage or pectin which adhered to the drying paper. The amount of carbon removed on the paper may be calculated from the difference between the initial carbon content of the discs and the final carbon recovered in the discs and solution plus the carbon value of the respiration and the volatile compounds evolved (equivalent to 7.5 per cent. of the carbon dioxide of respiration). Knowing the carbon removed by the blotting technique, its equivalent of starch, dry matter, or calories may be calculated since the parent substance was present in the initial discs in a form which was estimated as starch and, when the film was removed, the discs retained none of the carbon, dry weight, or heat of this substance (table VI).[12] The results of these calculations are set out in table X; in table XI the percentage recovery of the initial metabolites is given.

Reference to table XI and to the detailed figure 2 shows that the metabolic processes of potato discs have now been adequately comprehended and the fate of the principal metabolites estimated quantitatively. The account now rendered of the original carbohydrate and dry matter is accurate to 1 per cent. (except in two cases where the total discrepancy is 3 per cent.) despite the varied treatments to which the tissue was subjected and the diverse metabolic processes involved.

Source of carbon for protein synthesis

It is important to recognize that the balance sheet of carbohydrates is complete without any actual allowance for carbon which is drawn from sugar and built into the protein molecule. In other words, if the equivalent of the carbon content of the stored soluble nitrogen compounds is inadequate it must be supplemented from sources other than sugar (*e.g.*, organic acids which also disappear when protein is synthesized (**47**)).

[12] If the salivary extract used for starch hydrolysis also hydrolyzed the uronic-acid complex designated pectin, then from the standpoint of the balance sheets it would be immaterial whether the uronic-acid complex removed by blotting was synthesized from starch during the treatments or merely rendered mobile in the presence of oxygen, potassium bromide, and a temperature of 23° C., so that it accumulated as an adhesive film at the surface. Lacking the definite proof of this point for potato pectin it will be convenient to write of the pectin-like substance as though it were in fact synthesized during the treatment from the starch reserve. This can be justified from data on commercial apple pectin. A stronger salivary extract than that used for the starch hydrolyses (10 ml. saliva + 10 ml. water) yielded no more sugar after 24 hours incubation at 25° C. with 0.10 gm. of pectin than the original sample contained (0.010 gm. reducing sugar plus 0.005 gm. sugar inversion by 3 per cent. HCl for 15 min.).

TABLE X

ABSOLUTE RECOVERY OF INITIAL METABOLITES

Number	Conditions	Carbon recovered in discs + solution	Carbon evolved as CO_2	Volatile compounds	Total carbon recovered	Carbon removed as surface film on drying paper	Original dry weight of starch = carbon removed on drying paper	Starch previously accounted for‡	Total starch accounted for	Dry weight previously accounted for‡	Total dry weight accounted for	Original* calorific value of substance removed in drying paper	Calorific value previously accounted for‡	Total calorific value accounted for
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*kgm. cal.*	*kgm. cal.*	*kgm. cal.*
1	Dist. H_2O, air	2.080†	0.185	0.014	2.279	0.249	0.561	3.762	4.323	5.900	6.461	2.37	24.61	26.98
2	0.005 equiv. KBr, air	2.073	0.193	0.014	2.280	0.248	0.558	3.704	4.262	5.793	6.351	2.355	23.70	25.95
3	0.05 equiv. KBr, air	2.033	0.228	0.017	2.278	0.250	0.562	3.763	4.325	5.190	6.472	2.375	23.66	26.03
4	0.005 equiv. KBr, 3.80 per cent. O_2	2.415	0.083	0.006	2.504	0.024	0.054	4.255	4.309	6.417	6.471	0.288	26.03	26.26
5	0.005 equiv. KNO_3, air	2.124	0.256	0.019	2.399	0.129	0.291	3.967	4.258	5.296	6.587	1.230	25.83	27.06
6	0.005 equiv. $CaBr_2$, air	2.235	0.170	0.013	2.418	0.110	0.247	4.154	4.401	6.304	6.551	1.04	25.94	26.98
7	Initial tissue	2.528	.....	.....	2.528	.....	.....	4.286	4.286	.....	6.40	.....	25.80	25.80

* Starch = carbon lost.
Starch = 4228 cal. per gram.
† Based on replicate analyses of one sample only, not mean of two parallel batches as in other cases.
‡ See table V.

TABLE XI

PERCENTAGE OF RECOVERY OF INITIAL METABOLITES

Treatment		Recovery of original dry weight	Recovery of original starch	Recovery of original calorific value*
Number	Conditions			
		%	%	%
1	Dist. H_2O, air	101.0	101.0	105.0
2	0.005 equiv. KBr, air	99.3	99.5	100.2
3	0.05 equiv. KBr, air	101.0	101.0	100.7
4	0.005 equiv. KBr, 3.80 per cent. O_2	101.0	101.0	102.0
5	0.005 equiv. KNO_3, air	103.0	100.7	104.8
6	0.005 equiv. $CaBr_2$, air	101.0	103.0	104.5

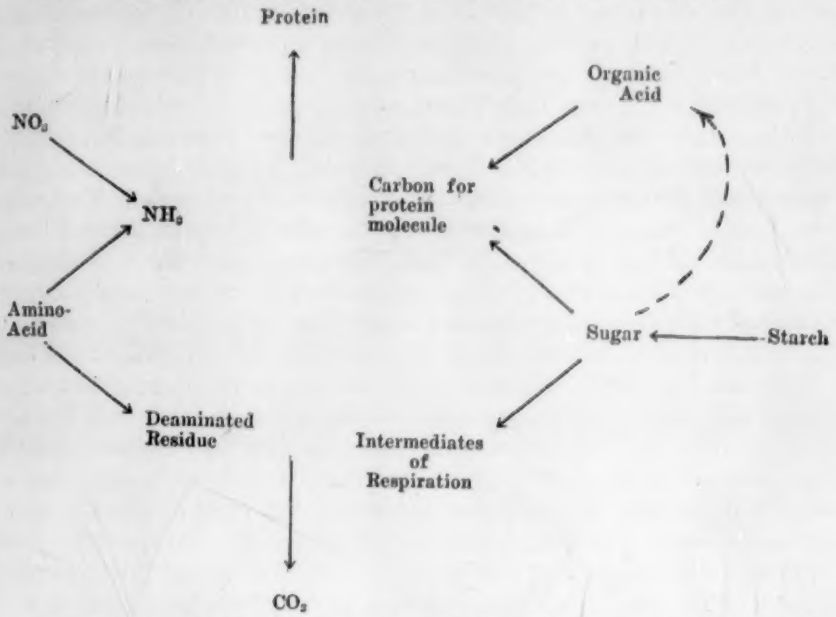
* Assuming all the heat of respiration $C_6H_{12}O_6 = 6CO_2 + 674{,}000$ cal. was absorbed by the surroundings.

Figure 1 implies that the same relationship exists between the nitrogen metabolism to protein and respiration whether the nitrogen is obtained direct from potassium nitrate or when the soluble nitrogen compounds of the cells are used. In the latter case the source of nitrogen also contributes carbon; in the former all the carbon involved is drawn from nitrogen-free reserves in the cells. The carbohydrate balance sheet (no. 5) is already complete although the nitrogen absorbed and converted to protein would require the carbon of approximately 3.5 per cent. of the total starch. Hence organic acids and not carbohydrate must be regarded as the probable source of carbon for the fabric of the protein molecule formed from nitrate and this strengthens the probability (see below) that these substances contribute the extra carbon necessary if synthesis proceeds from amino acids which are richer in nitrogen and poorer in carbon than the resultant protein.

There is, however, yet another apparent paradox. The connection between aerobic respiration and protein synthesis appears to be that deamination of amino acids liberates nitrogen-free residues and ammonia; the deaminated residues contribute to the carbon substrates of respiration and the ammonia forms protein with compounds derived in part, at least, from sugar and supplemented when necessary (*e.g.*, NO_3 culture no. 5) from organic acids. To preserve the appearance in the balance sheet that the carbohydrate carbon is accounted for apart from protein synthesis it is only necessary that the deaminated residues yield as much carbon dioxide as would otherwise be expected from that moiety of the protein molecule which is derived from sugar. Hence from the standpoint of the balance sheet the carbon content of the amino acids and the sugar which is built into the framework of the protein are interchangeable; it is immaterial which supplies the carbon for synthesis and which is converted to carbon

dioxide. It is clear, however that the *extra respiration stimulated by protein synthesis is more than could arise from the deaminated residues alone* and the excess must be due to an increased oxidation of sugar for which allowance has already been made in the carbohydrate balance sheet.

The relationships outlined above can be expressed as follows:



Though necessarily obscure as to details this scheme emphasizes that approximately $\frac{2}{3}$ of the respiration of potato discs in water is attributable to a respiratory component which is intimately connected with protein synthesis and the processes of nitrogen metabolism. The pace of this intricate piece of metabolic machinery may be accelerated (*e.g.*, by oxygen supply, potassium, or nitrate ions) or depressed (by low oxygen concentration or calcium ions) by factors which ultimately affect all the component parts of a closely integrated system even though their immediate effects may be localized.

Other implications of the metabolic balance sheet of potato discs

In the presence of a calcium salt (no. 6) the balance sheet shows an apparent recovery of 103 per cent. of the original starch. The titration curve and pH data (**47**) and this paper (table I) show, however, that in the presence of a calcium salt the metabolism of organic acids was stimulated and the apparent recovery of more than the original starch in the balance sheet suggests that some of the carbon dioxide evolved came from

sources other than carbohydrate. In potato discs in calcium solutions protein synthesis and its associated phases of respiration are depressed and the indications are that the metabolism of organic acids makes a relatively greater contribution to the total carbon dioxide production.

Table V shows the effect on the calorific value of the removal of a surface film of organic matter from the discs by the blotting technique. Correcting for this the outstanding losses of calorific value can be compared with the heat value of respiration assuming that all of the carbon dioxide was produced aerobically from sugar. Assuming that the heat of respiration disappeared from the tissue system, an account of the original calorific value is rendered in table X. Treatments nos. 4 and 6 favored reactions (the relative preponderance of anaerobically produced carbon dioxide in case of no. 4 and the oxidation of organic acid in case of no. 6) which clearly have a lower energy value than that upon which the heat value of respiration was calculated; this is consistent with an apparent recovery of original calorific value somewhat greater than 100 per cent. Treatments nos. 2, 3, and 5, which stimulated aerobic respiration, provided the conditions most favorable to the uptake of ions, and in these cases the energy balance sheet most nearly approached to 100 per cent., assuming that *all of the energy of respiration was dissipated as heat and absorbed by the surroundings.* Even so, the apparent recovery of calorific value tends to exceed 100 per cent. but this does not mean that some of the energy of respiration was retained by the discs but rather that the heat value of respiration was assessed too highly since aerobic respiration includes some carbon dioxide which arises from reactions with a lower energy value.

The somewhat surprising possibility that all of the energy value of respiration is dissipated as heat and, under isothermal conditions, is absorbed by the surroundings is not without precedent. Both FIFE and ALGERA[13] (**47**), using organisms which in some respects are more tractable for the energy measurements (Azotobacter and Aspergillus respectively), found that the energy of respiration reappeared quantitatively as heat. In the data on calorific value of potato discs there is no evidence that the great decrease in their heat content which occurs during salt uptake (**41**) is directly applied to processes (*e.g.*, salt accumulation) in which work is done. At most the heat measurements record the change in the total heat content of the discs whereas the free energy change of the system determines its capacity to do work. Heat losses in the discs which are not due to respiration have been traced to causes which are *unconnected with the performance of osmotic work.* Present methods cannot measure the energy utilized in salt absorption *per se* which would represent only a very small part of the total energy turnover which occurs in the tissue.

[13] ALGERA produced an energy balance sheet in which the discrepancy was only 0.5 per cent. of the total—an amount within the experimental errors.

The fact of metabolic activity in storage tissue under conditions conducive to salt absorption is, however, conclusively established; this can be appreciated at once from figure 2. The magnitude of these effects may occasion some surprise even though previous papers (**38, 39, 40, 41, 42, 43, 44, 45**) anticipated that the relationship between salt uptake and vital processes would involve all the attributes of growing cells. Potato discs, though composed of storage tissue which has been supposed by many to be free of such complications, carry out all the processes (other than photosynthesis) of which parenchyma is capable. The specific "salt effects" are superimposed upon a basic condition of active metabolism—determined by the variables oxygen, temperature, and disc thickness—which is a prerequisite for salt accumulation (no. 1). Starch hydrolysis, rapid respiration, protein synthesis from amino acids, the disappearance of organic acids from the sap (**47**), an increased content of reducing substances (ascorbic acid and glutathione), and the activity of the catechol oxidase system of the potato (which also has the property of de-aminating amino acids), are all prominent features of this system in which salt accumulation may occur. Almost all of these processes are, however, affected by the nature and concentration of the salt in the external solution. The outstanding result is that all these processes which are stimulated by oxygen (compare treatments nos. 2 and 4) are intensified by the presence of potassium and still further by nitrate, whereas they are depressed by calcium. The effects due to the ions (K, NO_3, Ca) become intelligible when it is realized that they operate through the nitrogen metabolism.

The parallelism between aerobic respiration and protein synthesis revealed by figure 1 is a striking corollary to the demonstrable parallelism between aerobic respiration and salt accumulation (**21, 37**). Whereas the mechanism of deamination of amino acids provides a credible link between protein synthesis and respiration the connection between both processes and salt accumulation is still obscure. Schemata of respiration, which refer (**6**) to cells which have lost their capacity for constructive metabolism or are limited in their exercise of it by external variables, cannot be applied to the respiration of potato discs if they ignore the striking parallelism between the utilization of amino acids in protein synthesis and respiration which is so conspicuous a feature of the metabolism of these cells which are still able to grow and accumulate salts. There are several features of the schema described by GREGORY and SEN (**18**) which seem to apply to potato discs but these will be discussed in detail at a later date.

Specific reactions causally connected with salt accumulation remain elusive. Protein synthesis and aerobic respiration are intensified or depressed according as the nature of the salt, its concentration, or the oxygen conditions facilitate, or depress, absorption; and it is clear that these are

the aspects of metabolism which are most closely linked with salt uptake. The suggestion now is that water absorption is also brought about by metabolic processes in a manner not readily explained by any simple osmotic theory. As yet one can only conclude that, not one, but the summation of *all* the metabolic and biochemical processes described—processes dependent upon oxygen—constitutes that "dynamic machinery" which is essential for salt accumulation. The problem raised, however, is not only how these processes in living cells are directed to the actual accumulation of salts but *why they constitute such an apparently wasteful method of applying metabolic energy to the performance of useful work.*

Summary

The effects of six selected salt and oxygen treatments during 70 hours at 23° C. on the respiration and metabolism of potato discs have been investigated.

Those salt treatments (KNO_3, $CaBr_2$) in which uptake of the anion exceeds that of cation cause an increase in the bicarbonate concentration and alkalinity of the external solution.

In aerated solutions potassium salts stimulated, and calcium salts depressed, water absorption in a manner not wholly explicable by osmotic phenomena. This suggests that a re-investigation of water absorption and its relation to metabolic processes would be profitable.

At oxygen tensions such that respiration is not limited by oxygen the respiratory behavior of potato discs is determined by the salts in the external solution. When the concentration of potassium bromide in the external solution is increased, the respiration rate is also increased. In the presence of calcium bromide the respiration is less than in distilled water. At the same equivalent concentration potassium nitrate increases respiration more than does potassium bromide.

The effect of low oxygen concentrations on respiration has been confirmed.

All the treatments cause an increased sugar concentration relative to that in the initial tissue. The salt and oxygen treatments which stimulate respiration produce a low, residual sugar concentration. The concentration of sugar does not regulate the respiration rate.

At a low oxygen concentration (solution in equilibrium with 3.80 per cent. O_2) at which accumulation of bromide was depressed, synthesis of protein from amino acid was also limited by oxygen lack. In aerated distilled water protein synthesis occurs and this is increased by the presence in the external solution of potassium bromide but is decreased by calcium bromide. Dilute potassium nitrate produced the greatest observed effect on protein synthesis.

An approximately linear relationship was found between the amount of protein synthesized and the quantity of carbon dioxide respired.

Approximately $\frac{1}{3}$ of the respiration of discs in aerated distilled water is produced independently of nitrogen metabolism. This component of the total respiration is not affected by salts or oxygen concentration. Two thirds of the respiration of discs in distilled water arises from a component of respiration which is linked to protein synthesis from amino acids, is affected by salts and oxygen concentration and in which the oxidase system is involved.

The effects of inorganic ions on respiration are parallel to their effect on protein synthesis. Potassium and nitrate ions intensify, while calcium retards, the effect of oxygen on respiration.

The characteristic effects of ions on respiration and synthesis are exerted at oxygen tensions at which their absorption occurs and the reactions of the ions observed therefore constitute an integral part of the machinery of salt absorption.

A preliminary balance sheet of metabolites showed that significant amounts of carbon, dry weight, carbohydrate, and calorific value were not accounted for when the tissue respired in aerated water or potassium bromide solution.

The discrepancies in the balance sheet proved to be caused by the transfer of organic substance from the surface of those discs which had been in aerated potassium bromide solution or distilled water to the drying papers used to remove surface water. The losses named were real at high oxygen tensions, did not occur at low oxygen tension, and were small in the presence of calcium salts.

The surface film removed was formed at the expense of the starch fraction and consisted of a complex rich in uronic acid. If the uronic acid removed from the discs by blotting is expressed as pectin the concomitant loss of carbon can be accounted for and also a large part of the loss of dry weight.

It is now believed that losses of magnesium referred to in an earlier paper (**38**) are attributable to a similar cause.

Volatile organic compounds are produced by potato tissue under the conditions of these experiments only in small amount. Such compounds appear in the flowing gas stream and small amounts are evolved when the tissue is dried. The carbon value of the total production of volatile compounds is of the order of 7.5 per cent. of the total respiration.

A revised balance sheet embodies corrections for all the above processes and summarizes satisfactorily the principal processes which occur in potato discs under the conditions conducive to salt uptake and the effects of salts and oxygen upon them at 23° C.

The amino acids and sugar supply the carbon for the protein molecule and the extra respiration entailed in its production. In the fixation of nitrate nitrogen, carbon is derived from other sources (organic acids). Organic acids disappear during protein synthesis from amino acids and probably contribute here also to the carbon framework of the protein molecule.

Experimental difficulties prevent an estimate of the energy involved in salt uptake *per se*. The implication of the data is that all of the energy liberated by respiration is absorbed by the surroundings as heat.

The significance of the results relative to current views on respiration and salt uptake is discussed.

The work here described represents the results of an investigation started in the Division of Plant Nutrition, University of California, in 1933–1934. During this period the senior author received leave of absence from the University of Leeds for which he is indebted to PROFESSOR J. H. PRIESTLEY. The work has been continued since 1934 in both the laboratories mentioned. For the continued help supplied by the Division of Plant Nutrition we wish to express our thanks and also for the personal contribution of PROFESSOR D. R. HOAGLAND to our work. It should be recognized that after the preliminary stages Dr. P. R. STOUT, a member of the Division of Plant Nutrition of the University of California, was almost solely responsible for the calorimetric work and many of the quantitative determinations in this paper whereas DR. C. PRESTON contributed the experimental data dealing with volatile compounds and the loss of dry matter by blotting the discs. For the determinations of the nitrogen and carbohydrate fractions we have to thank Dr. W. Z. HASSID of the Division of Plant Nutrition.

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EFFECT OF SOIL MOISTURE ON GROWTH AND TRANSPIRATION IN *HELIANTHUS ANNUUS*

EMMETT V. MARTIN

(WITH FOUR FIGURES)

Introduction

Work done previous to 1913 concerning the effects of soil moisture on growth and transpiration of plants is summarized in a paper by BRIGGS and SHANTZ (**2**). Practically all of these earlier workers found that there was an increase in growth with increasing holard over most of its range, although growth was somewhat reduced in soil that was nearly or completely saturated. Water requirement usually increased regularly with holard, but in a few cases very low values of soil moisture gave higher water requirements.

TUMANOW (**16**) has grown sunflowers in small containers holding six kilograms of dry soil. The holard of one set of plants was maintained near 60 per cent. of the maximum capacity of the soil, while that of another set was allowed to vary between this value and the wilting point. The experiment was continued throughout the life cycle, during which time the test plants experienced sixteen wiltings. At the end of the series the control plants had about twice the dry weight of the others, but only 20 per cent. greater water requirement.

TUMANOW also grew bean plants to flowering in pots containing 3500 gm. of dry soil. One set (the control) was maintained at a holard of 60 per cent. of the capacity of the soil, while the other two sets were started with holards of 50 per cent. and 40 per cent., but neither of the latter was given any additional water during the growing period. At the end of the series, the average holards of these last two groups were 24 and 30 per cent. respectively. The dry weights were 11.66 grams for the control set, 5.43 for the 50 per cent. group, and 2.21 for the 40 per cent., while the water requirements were 136, 108, and 86 respectively.

CLEMENTS and LONG (**3, 4**) grew sunflowers in metal containers eight inches in diameter and ten inches tall with average holards maintained approximately at 14, 18, 26, and 35 per cent. of the dry weight of the soil, and found that growth and water requirement increased regularly with holard. This was true in the sun as well as in the shade, although in low light values the influence of holard on the water requirement was more marked.

PESSIN (**14**) grew seedlings of *Pinus palustris* and *Pinus caribaea* for six months in quart containers with holards equal to 5, 10, 15, 25, and 35 per cent. of the dry weight of the soil. The dry weights of both species

increased with holard to 25 per cent., but those in 35 per cent. were smaller. The water requirement of *Pinus caribaea* rose regularly from 364 for the 5 per cent. group to 1163 for the 35 per cent., while that of *Pinus palustris* had a maximum at 15 per cent. holard. In neither species was there any evidence of an increase for the very low values of available water.

BRIGGS and SHANTZ (**2**) and SHANTZ (**15**) have raised the objection to much of the earlier work on the effects of different degrees of soil moisture on growth and transpiration that it is impossible to maintain a mass of soil at a uniform low holard, because the small amounts of water added from time to time are insufficient to moisten the entire mass of soil.

VEIHMEYER (**17**) has done a great deal of work relating to the penetration of water into soil, and has found that water applied to the surface of soil penetrates only to such a depth that the soil through which it passes is raised to its field capacity. Further penetration is exceedingly slow, and ordinarily is of little consequence to plants. If a plant growing in a container has reduced the holard to the wilting point, the only way in which the water-content of the entire mass of soil can be raised is to add enough water to bring all of the soil to its field capacity. Addition of less than this amount will leave the holard of some of the soil in the bottom of the container unchanged. In such a case, the plant would be forced to grow in a smaller volume of soil than another plant in a similar container with higher average holard.

In conducting studies of the effect of soil moisture on plants, VEIHMEYER (**17**) recommends avoiding this difficulty of soil-water distribution by adding at each irrigation enough water to bring all the soil to its field capacity, but allowing different plants to reduce the holard to different degrees of dryness before again adding water. This procedure permits each plant to have water available throughout the entire soil mass, but the various plants would be working through different ranges of soil moisture. Using this method with prune trees, he has found that growth and rate of extraction of water from the soil are not appreciably affected over the range of holard from the field capacity to approximately the wilting percentage, results which are not at all in accordance with previous work.

HENDRICKSON and VEIHMEYER (**5, 6, 7, 8**), experimenting in the field with prune, peach, pear, and walnut trees and grape vines, have obtained similar results, and have arrived at the conclusion (**5**) that "trees either *have* readily available water or *have not*." BECKETT, BLANEY, and TAYLOR (**1**), working in citrus and avocado orchards, arrived at a similar conclusion.

MAGNESS, DEGMAN, and FURR (**13**) have found that the behavior of stomata of apple trees may be affected when the moisture content of the whole root zone is apparently considerably above the wilting percentage, while at least part of the root zone on soils of medium or light texture is

usually at or near the wilting percentage before variations in the growth rate of the fruit can be detected.

Very different results have been obtained by LEWIS, WORK, and ALDRICH (**9, 10**) and by WORK and LEWIS (**19**) with pears on a heavy clay soil. They found that the fruit growth rate was reduced whenever the soil moisture was lowered below 70 per cent. of the available capacity.

An explanation of these apparently contradictory results has been advanced by MAGNESS (**12**) and by LEWIS, WORK, and ALDRICH (**10**). They suggest that, in those cases in which trees have suffered from water-shortage when the soil moisture was well above the wilting percentage, the trees were growing on heavy soils with slow capillary movement of water and poorly distributed root systems. As a result, the soil in immediate contact with the absorbing roots may be at or near the wilting percentage while the average in masses large enough to be sampled may be well above this point. On the other hand, experiments which have shown that soil moisture is equally available from the field capacity to about the wilting percentage have been with trees growing on moderate to light textured soils in which the root distribution is usually much more complete and capillary movement more rapid than in heavy soils. According to this hypothesis, in all cases of water-shortage the soil moisture in the immediate vicinity of the absorbing roots is at or near the wilting percentage, even though only a short distance away it may be well above this point. Failure of the tree to obtain water under these circumstances is presumed to be due to the relatively great spacing of the roots and slow capillary movement of water through the soil.

The present investigations were undertaken with the purpose of obtaining further information concerning the effect of soil moisture on growth and transpiration of *Helianthus annuus*, using a light, fertile, sandy loam in order to permit as great a degree of root growth and capillary movement of water as possible.

Methods

For all experiments reported in this paper, plants of the Russian Mammoth variety of *Helianthus annuus* obtained from commercially produced seed were used. The seeds were soaked in water 24 hours before being planted in special pots made of asphalt felt (tar paper) rolled into a cylinder about 2 inches in diameter and 8 inches long. With this type of pot, it was possible to transfer the seedlings to the phytometer cans without disturbing the roots. Four seeds were planted in each pot, and the seedlings were selected for uniformity at the time the cans were sealed.

For all series, galvanized iron cans 13 inches in diameter and 22 inches tall with removable lids were used. The soil employed was a rather light, sandy loam with a field capacity of 18 per cent. and a permanent wilting

coefficient of 7 per cent. of the dry weight. All the soil used in any particular series was thoroughly mixed as a whole in order to have as great a degree of uniformity among the cans as possible.

For series I–III, the soil for each can was weighed out and mixed in a metal pan with about 100 grams of the commercial fertilizer Vigoro. It was finally run through a 6×6 mesh screen and packed firmly in the cans. The seedlings were then transplanted and enough water was added to bring the soil to its field capacity. As soon as the seedlings were tall enough to project above the lid, the opening in the center of the lid was sealed around the stem of the plant with modelling clay. This seal excluded rain and reduced evaporation satisfactorily. For each of these three series, 12 cans were used. They were divided into two sets of 6 each and given different treatments as follows: one group, called the control, was watered frequently in order to keep the holard above 14 per cent. The other group, called the test set, was watered only when the holard had been reduced to about 10 per cent., and then was given enough water to bring the soil back to its field capacity. All water was added to the surface of the soil. Samples indicated that at least 12 hours were required for water to penetrate to the bottom of the can, so that ordinarily watering was done in the late afternoon in order to allow penetration during the night when transpiration was low. On this account, it was necessary to discontinue these series when the control plants were nearly to the point of needing water more than once each day.

For series IV–V, the procedure was different in that the soil was mixed at a predetermined holard at the time of filling the cans, and no water was added at any time during the series. Four groups of 6 cans each were employed, with average initial holards of approximately 11, 14, 17, and 20 per cent. of the dry weight of the soil. The soil for each can was mixed in a flat metal pan with about 100 grams of Vigoro and enough water to bring the soil to the desired holard. The soil was finally run through a 6×6 mesh screen and pressed down firmly in the can. For the group with 20 per cent. holard, the soil was mixed at 14 per cent. and was brought to its proper value by the addition of water after it was placed in the cans. The seedlings were transferred to the cans immediately after filling. A circular piece of tar paper cut to fit the inside of the can was placed on top of the soil. This paper had a 2-inch circular hole in the center for the plant. Both this hole and the gap around the edge of the paper were sealed with modelling clay. The lid of the can was put on at this time, and as soon as the plant projected through, the hole in the lid also was sealed around the stem of the plant with clay as in the other series. This seal was very effective in controlling evaporation from the soil and in excluding rain. Cotton placed around the stem of the plant prevented the seal from getting

too hot due to exposure to radiation. The cans were weighed once each week on a platform scale having a sensitivity of 1/100 pound. Both these series were brought to a close when the holard of the 11 per cent. group had been reduced to about 9 per cent.

At various times throughout the series, measurements of stem height, stem diameter, and leaf area were made. The leaf areas in square centimeters were determined by multiplying the products of length and width of the leaves by the factor 1.34. This procedure yielded the sum of the areas of both sides of the leaf. At the end of a series, final measurements were taken and the plants were dried in an electric oven at 65° C. The roots were washed out of the soil by a stream of water from a nozzle.

Measurements of the size and degree of opening of the stomata on the lower surface of the leaves were made from strips of epidermis preserved in absolute alcohol according to the method of LLOYD (**11**).

Values of soil moisture during the series were calculated from the weights of the cans and the initial known holard, although occasional samples were taken in order to correct for the changing weight of the plant. For this purpose, a circular hole about 4 inches in diameter was dug with a trowel near the edge of the can, and samples of approximately 200 grams each were taken from the inner side of the hole at depths of 2, 8, 14, and 20 inches from the top surface of the soil. This procedure gave samples at points about midway between the center and edge of the soil mass. This method of sampling disturbed the root system some, but there was no apparent effect on the shoot, either in appearance or in growth rate. No can was sampled more than once. Roots were screened out of the sample before drying.

The validity of the average of a group of plants depends upon having all individuals of a group in the same environment. In the case of soil moisture studies, all plants of a given group should be growing in soil with the same holard. On account of the fact that different plants use water at different rates, this condition is possible only within certain limits. In the experiments reported in this paper, the cases were very rare in which the average holard for any plant deviated more than 1 per cent. from the average of its group, and many of them were within 0.5 per cent. These limits seemed to be sufficiently narrow to justify averaging the results of the individuals in the various groups.

Results

SERIES I–III

The values of holard, leaf area, and transpiration rate per unit leaf surface for series II and III are shown graphically in figure 1. As the graph shows, there was a noticeable difference in the leaf areas of the test and

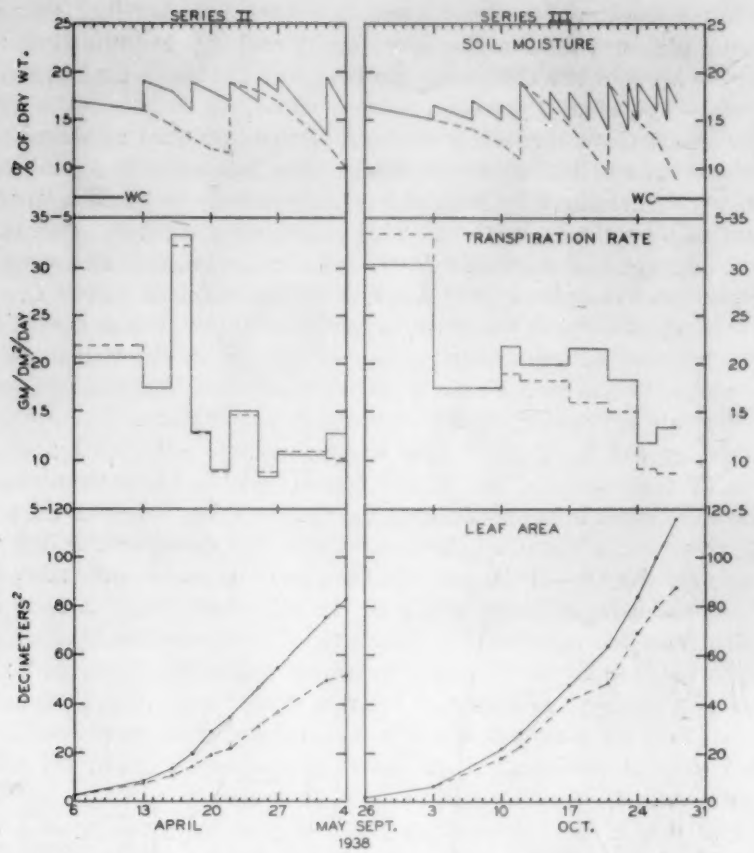

FIG. 1. Variation in soil moisture, transpiration rate, and leaf area during series II and III. Values for the control and test groups are indicated by the solid and broken lines respectively.

control plants by the time the average holard of the former had been reduced to 13 per cent., and the difference was marked at 11 per cent., when the test set was given its first watering. At this point in series II, the control group had a leaf area 58 per cent. greater than that of the test set, while for all three series the average difference was 41 per cent. In all three series the differentiation began almost as soon as the test set was given its first watering.

It was possible that, during the period immediately following this watering of the control set, the plants of the test set might be using the water only from the soil in the upper part of the cans, reducing this portion to near the wilting coefficient but leaving the lower part moist, thus producing the observed difference in growth. In order to obtain some information

on this point, soil samples were taken in series II on April 22, just before watering the test set. The holard at depths of 2, 8, 14, and 20 inches in the test set was found to be 10.1, 10.6, 11.1, and 10.8 per cent., respectively. On Oct. 10 in series III, samples at the same depths showed holards in the test set of 11.1, 11.6, 13.4, and 11.9 per cent. Although this does not demonstrate that the soil immediately in contact with the roots was not at or near the wilting coefficient, it does show that these plants removed the moisture from all regions of the soil in a remarkably uniform manner.

The increase in leaf area of the plants in series II and III during consecutive intervals of the growing period in percentage of the values at the beginning of the intervals is given in table I. In both series, as the holard

TABLE I

INCREASE IN LEAF AREA OF THE PLANTS IN SERIES II AND III DURING CONSECUTIVE INTERVALS OF THE GROWING PERIOD IN PERCENTAGE OF THE VALUE AT THE BEGINNING OF THE INTERVAL IN QUESTION

SERIES II

INTERVAL	APR. 6 APR. 13	APR. 13 APR. 16	APR. 16 APR. 18	APR. 18 APR. 20	APR. 20 APR. 22	APR. 22 APR. 25	APR. 25 APR. 27	APR. 27 MAY 2	MAY 2 MAY 4
Control	233	54.5	43.9	46.5	18.8	35.1	16.4	37.6	10.0
Test	218	39.2	34.6	32.4	12.8	41.2	17.9	36.4	2.6

SERIES III

INTERVAL	OCT. 3 OCT. 10	OCT. 10 OCT. 12	OCT. 12 OCT. 17	OCT. 17 OCT. 21	OCT. 21 OCT. 24	OCT. 24 OCT. 26	OCT. 26 OCT. 28
Control	224	30.9	68.6	33.6	30.8	19.5	16.0
Test	177	24.2	82.4	16.4	40.2	16.0	9.9

of the test set decreased, the rate of increase of leaf area of this group dropped off with respect to that of the control; for periods immediately after watering, the rate of increase rose above that of the control. For periods just previous to watering, the rate of growth of the test group (as measured by leaf area increase) varied from about one-half to three-fourths that of the control; afterward the rate rose to about 1.2 times the latter. This same behavior was noted in stem diameter, although it was less marked and less regular, presumably on account of less accurate measurements. This effect is probably due largely to recovery of turgor, although it is possible that there is an increase in the rate of assimilation, as TUMANOW (**16**) claims.

The rate of transpiration per unit of leaf surface was not entirely consistent in its relation to holard. In series II the rate was not affected until the holard had dropped below 11 per cent., but in series III the effect was noticed at about 12 per cent. This difference is slight, and is probably due to the somewhat higher transpiration rates in the latter case. In series III, after the initial reduction in transpiration rate with low holard, the test

plants never recovered equality with the controls. This effect was not evident in series II, since the first period of low holard did not affect the transpiration rate. In series I, the rate of transpiration was reduced about 20 per cent. at a holard of 11 per cent., but it regained equality with the controls after watering. TUMANOW (16) found that his sunflower plants after recovery from wilting had a transpiration rate well above that of the controls.

Measurements of the diurnal variation of stomatal opening on several occasions during series II gave the following results. On April 21, a time of relatively low holard of the test set, the stomata of the test set closed partially about 2:00 P.M. while those of the control plants remained open. On April 23 and 27, times of high holard and both cloudy days, stomata remained open all day on both groups. On May 3, at low holard of the test set, stomata remained nearly closed all day on the test set and practically wide open on the control. This was the only period during which the transpiration rate of the test set was appreciably reduced below that of the control. It was also the only period during which the test plants showed any visible signs of flaccidity of the leaves, although even in this case the wilting was very slight.

For each of the three series, the plants were allowed to grow until those

TABLE II

SUMMARY OF DATA OBTAINED FROM SERIES I–III. ALL MEASUREMENTS ARE AVERAGES OF ALL PLANTS IN EACH GROUP

ITEM	SERIES I		SERIES II		SERIES III	
	TEST GROUP	CONTROL GROUP	TEST GROUP	CONTROL GROUP	TEST GROUP	CONTROL GROUP
Number of plants in group	4.0	5.0	6.0	6.0	6.0	6.0
Stem height (cm.)	75.0	87.0	68.0	65.0	73.0	83.0
Stem diameter (cm.)	2.0	2.33	1.89	2.30	1.85	2.48
Leaf area (dm.^{2})	63.3	101.0	51.5	82.8	87.6	116.0
Fresh weight of shoot (gm.)	413.0	654.0	320.0	511.0	424.0	688.0
Dry weight of shoot (gm.)	49.4	70.8	37.4	53.1	53.0	73.5
Dry weight of roots (gm.)	11.4	12.3	9.82	14.2	11.5	13.5
Dry weight, total (gm.)	60.8	83.1	47.2	67.3	64.5	86.9
Shoot/root ratio	4.53	5.97	3.83	3.93	4.76	5.48
Percentage of water in shoot	88.0	89.2	88.3	89.6	87.5	89.4
Total transpiration (kg.)	11.49	18.88	10.97	16.16	15.39	22.70
Water requirement (gm./gm.)	189.0	227.0	235.0	240.0	239.0	267.0
Number of stomata per mm.^{2}	219.0	153.0	256.0	213.0	367.0	344.0
Thickness of leaf (microns)	.....	.....	249.0	316.0	221.0	246.0
Palisade (percentage of total thickness)	.....	.....	49.0	50.0	55.0	55.0
Date of planting seeds	Jan. 15, 1938		Feb. 24, 1938		Sept. 8, 1938	
Date of harvesting plants	April 13. 1938		May 6, 1938		Oct. 28, 1938	

in the control group were nearly to the point of needing watering more than once each day. At this time the series were discontinued and final measurements taken. The results are given in table II, and those from series III are shown in figure 2. For all three series, the control plants

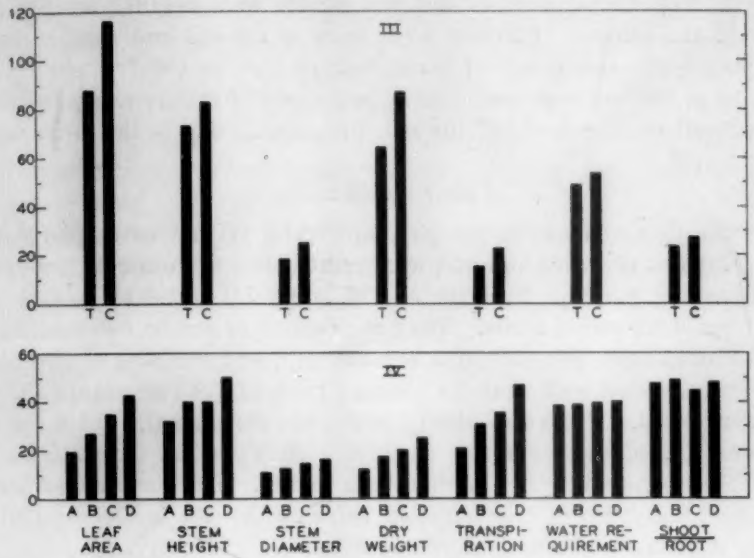

FIG. 2. Final measurements taken in series III and IV. The ordinate represents leaf area in square decimeters, stem height in centimeters, stem diameter in millimeters, dry weight in grams, transpiration in kilograms for series III and hectograms for series IV, water requirement in grams per gram $\times \frac{1}{3}$, and shoot-root ratio $\times 5$.

had the greatest stem diameter, leaf area, dry weight of shoot and roots, shoot-root ratio, percentage water in shoot, total transpiration, and water requirement.

Statistical tests according to the method of "Student," as outlined by FISHER, show that for all three series the differences in growth in terms of stem diameter, leaf area, and total dry weight are all significant (odds greater than 99:1). Similar results were obtained for water requirement in series I and III, but not in II. The odds are only about 5:1 that differences in stem height and shoot-root ratio in series I and III are real, but in II they are still less.

Series II appeared to be different from the others in that the shoot-root ratio, transpiration rate per unit leaf area, stem height, and water requirement were affected little if any by the different treatments. It might be noted in this connection that in the two series in which the transpiration rate per unit area was markedly affected, the water requirement was also reduced considerably, while in the other series neither was altered much.

Leaf sections were made in series II and III for measurements of the thickness of the leaf and the palisade tissue. In both series, the thickness of the leaves of the control plants was greater than that of the test plants, but the proportion of the leaf composed of palisade was the same for both sets. The epidermal cells of the test plants were slightly smaller than those of the control. Stomata were more numerous and smaller in the test plants, the size being in inverse proportion to the frequency. The anatomy of the leaf appeared to be largely a case of failure to expand under the reduced water-content of the soil, presumably due to the lower turgor of the leaves.

Short-period series

At the time of starting the plants of series III, six extra plants were put in cans of the same size and were treated like the controls throughout their growing period. Near the end of series III, these six plants were used for a short-period series. They were standardized by determining the transpiration rates per unit area for one day, and two sets of two plants each were selected such that the average rates of the two groups for that day were equal. These two groups were then considered to be a test and a control group, and for about ten days were treated in the same manner as the other plants in series III had been earlier. The variation of holard, leaf area, and ratio of transpiration rates of the test and control plants during the ten-day period is shown in figure 3.

The first portion of the transpiration curve shows considerable fluctuation about the ratio unity, but statistical tests show that this much variability is to be expected when only two plants are used in each set. Under these conditions, differences of about 20 per cent. in the transpiration rate per unit area are ordinarily detectable with two plants per set.

The ratio of the transpiration rate of the test set to that of the control was not appreciably altered until the holard of the former had been reduced to approximately 11 per cent.; at this point the ratio was about 0.8. Below this value of holard, the ratio dropped rapidly, reaching a value of 0.5 at 8 per cent. After the test set was watered, its rate of transpiration rose to equality with that of the control. The ratio was maintained above 0.8 until the holard of the test set had again dropped to 10 per cent., but it was reduced to 0.5 at 8 per cent. As before, watering led to a resumption of normal transpiration rate. Tumanow (**16**) reported an increase in the transpiration rate of sunflower plants after recovery from wilting above that of plants which have not wilted, but no such effect was noted in the experiments reported here.

Stomata were examined at each time of weighing the plants, and the degree of opening was found to be affected by holard only when the transpiration rate was reduced. Through the first period of falling transpira-

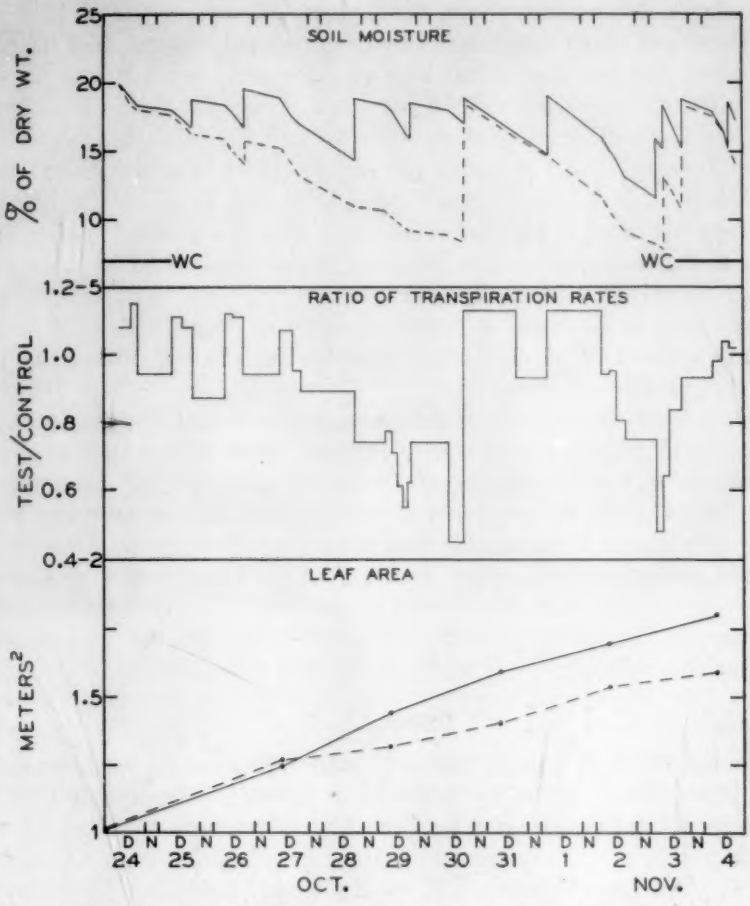

FIG. 3. Variation in soil moisture, ratio of transpiration rates of test and control groups, and leaf area during the short-period series.

tion rate, the stomatal opening of the plants of the test set became smaller and smaller until it appeared to be practically zero on Oct. 30, when the ratio was about 0.5. Since the plants were watered that evening, the stomata behaved the next day like those of the control set. At about noon on Nov. 2, when the transpiration ratio had again reached 0.8, the stomata of the test set began to close. They failed to open to more than mere slits the next morning. The plants were watered at 10:00 A.M. on this day; by noon the stomata were beginning to open, and at 4:00 P.M. they were as wide open as normally. In every case of reduced transpiration rate and closure of stomata there was a noticeable flaccidity of the leaves. This effect was slight at a transpiration ratio of 0.8, but there was marked wilting at a ratio of 0.5.

As in the other series above, these plants show an apparent increase in the rate of leaf growth after recovery from reduced turgor. For the period Oct. 29–31, the increase in leaf area of the control set was 10.4 per cent., while that of the test set was 6.9 per cent. From Oct. 31 to Nov. 2, after watering the test set, the values were 6.6 and 9.6 per cent., respectively. This difference is very probably not due to errors in measurement of leaf area, since comparisons of this method with that of measuring the areas of blueprints with a planimeter indicate that the probable error in the mean of the leaf areas of two plants of the size employed in this series is about 1.5 per cent. Since this degree of error appears to be caused largely by variations in the shape of leaves on different plants, it is probable that measurements of increases in leaf area for the same plants can be made with still greater accuracy.

One possible explanation of this phenomenon is that there is an increase in the rate of assimilation of plants recovered from wilting with consequent increase in rate of expansion of leaves. TUMANOW (**16**) presents data which indicate that such is the case, although the data are not convincing.

A more logical hypothesis is that more than 18 hours were required in this case for complete recovery of turgor of the leaves, although sunflower plants ordinarily apparently recover turgor within two or three hours after watering. Since this explanation cannot be ruled out, the data cannot be taken as clear evidence of anything more than this effect.

SERIES IV–V

The variation of holard, leaf area, and transpiration rate per unit of leaf surface during series IV is shown in figure 4. For the first week of the series, the transpiration rate of set A was higher than that of the other sets; however, statistical tests indicate that the differences are not significant. The same relation held for the second week, but for succeeding periods the rate of the sets with more water was greater than that of those with less. Numerous statistical tests show that differences in transpiration rate of approximately 15 per cent. are to be regarded as significant (odds 19 : 1). For the last three weeks of series IV, differences equal to about half the extremes shown in the graph satisfy this criterion. A test of the difference between the rates of sets A and D for the week of Dec. 22–29 gave odds of more than 99 : 1 for significance.

The manner in which the average holard for the different groups varied during the series is shown in the upper portion of figure 4. However, in this type of experiment it is possible that the roots might have been growing in the upper part of the soil mass only, reducing the holard of this region to a low value, with the result that the average holard obtained from calculations would not indicate the true moisture conditions under

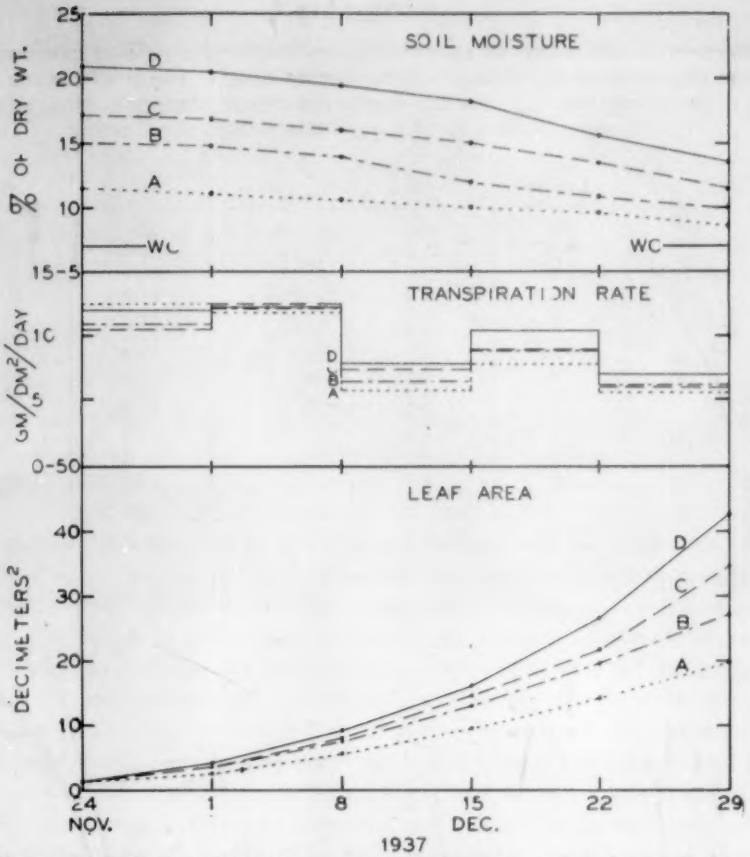

FIG. 4. Variation in soil moisture, transpiration rate, and leaf area during series IV. The values for the four groups with different initial holards are indicated by the letters A, B, C, and D.

which the plants were growing. In order to obtain some information concerning the distribution of moisture in the cans at various times during the series, samples were taken on Nov. 10, Dec. 22, and Dec. 29 at depths of 2, 8, 14, and 20 inches (bottom of the can), and the values obtained are recorded in table III. In all four sets, the tendency to maintain uniformity of holard at all depths is remarkable. Set D was initially given enough water to bring the average holard to greater than field capacity, with the result that there was an initial difference of 6.2 per cent. between the 2-inch and 20-inch levels. By Dec. 22, however, this difference had been reduced to 2.4 per cent. In the other three sets, the difference between top and bottom of the soil mass was less than 1 per cent. In none of the groups was there any evidence that the average holard of the upper parts

TABLE III

PERCENTAGES OF SOIL MOISTURE AT DIFFERENT DEPTHS IN THE CANS AT THE BEGINNING AND NEAR THE END OF SERIES IV. THE LETTERS A, B, C, AND D AT THE HEADS OF THE COLUMNS INDICATE THE GROUPS FOR WHICH THE INITIAL HOLARD WAS APPROXIMATELY 11, 14, 17, AND 20 PER CENT., RESPECTIVELY

DATE	DEPTH	A	B	C	D
	in.	%	%	%	%
	2	11.0	14.7	16.3	18.6
Nov. 10, 1937	8	11.8	15.1	17.0	19.8
	14	11.8	15.3	17.1	20.4
	20	11.9	15.6	18.3	24.8
	2	8.1	10.4	13.4	14.4
Dec. 22, 1937	8	8.7	11.1	13.6	14.8
	14	9.7	11.2	13.9	15.4
	20	9.2	10.5	13.2	16.8
	2	7.8	9.3	10.9	12.7
Dec. 29, 1937	8	9.8	10.2	11.5	12.9
	14	8.0	10.9	12.3	13.0
	20	8.7	9.1	10.9	15.1

of the soil mass had been reduced more than 1 or 2 per cent. before roots had penetrated to the bottom of the can.

In series V, soil samples were taken every week throughout the growing period, with results entirely in agreement with those of series IV. There appeared to be very little difference in rate of removal of water from different parts of the soil mass. Apparently the explanation of this phenomenon is that the roots were fairly well distributed throughout the soil mass by the time the plants had withdrawn enough water from the soil to reduce the average holard 1 or 2 per cent.

The variation of holard and leaf area during series V was similar to that in series IV, but the transpiration rates per unit leaf area behaved differently. Throughout the series the rate of transpiration of set A was from 10 to 15 per cent. greater than that of set D, with the other two sets intermediate, an order just opposite to that in series IV. This difference in behavior is presumably due to the fact that the plants in series V were much smaller at the end than were those in series IV, and accordingly had used much less water with less reduction in holard.

At the time the series were brought to a close, final measurements were taken, and the plants were cut down and dried in an electric oven at 65° C. The values obtained are given in table IV, and those from series IV are shown graphically in figure 2. In both series greater holards resulted in increased height, stem diameter, leaf area, dry weight of shoot and roots, percentage of water in the shoots, and total transpiration. There appeared to be no significant difference, however in shoot-root ratios. Water requirement behaved differently for the two series, increasing slightly with holard in series IV but decreasing in V.

TABLE IV

SUMMARY OF DATA OBTAINED FROM SERIES IV–V. ALL MEASUREMENTS ARE AVERAGES OF ALL PLANTS IN EACH GROUP. THE LETTERS A, B, C, AND D AT THE HEADS OF THE COLUMNS INDICATE THE GROUPS FOR WHICH THE INITIAL HOLARDS WERE APPROXIMATELY 11, 14, 17, AND 20 PER CENT., RESPECTIVELY

ITEM	SERIES IV				SERIES V			
	A	B	C	D	A	B	C	D
Number of plants in group	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Stem height (cm.)	32.0	40.0	41.0	51.0	16.0	18.5	26.8	29.2
Stem diameter (cm.)	1.09	1.24	1.44	1.63	0.64	0.78	1.04	1.20
Leaf area ($dm.^2$)	19.7	27.0	34.7	42.7	5.92	9.70	19.5	25.0
Fresh weight of shoot (gm.)	74.0	120.0	154.0	201.0	18.9	33.2	77.2	93.2
Dry weight of shoot (gm.)	11.0	16.0	18.3	22.9	2.83	4.37	9.21	10.25
Dry weight of roots (gm.)	1.16	1.63	2.06	2.38	0.55	0.77	1.64	1.95
Dry weight, total (gm.)	12.2	17.6	20.4	25.3	3.38	5.14	10.85	12.20
Shoot/root, ratio	9.48	9.82	8.88	9.61	5.20	5.71	5.66	5.42
Percentage of water in shoot	85.1	86.7	88.1	88.6	85.1	86.8	88.1	89.0
Total transpiration (gm.)	2100.0	3110.0	3585.0	4675.0	785.0	1169.0	2408.0	2685.0
Water requirement (gm./gm.)	174.0	177.0	177.0	186.0	236.0	227.0	223.0	221.0
Number of stomata per $mm.^2$	339.0	308.0	262.0	185.0	394.0	325.0	285.0	228.0
Thickness of leaf (microns)	175.0	175.0	203.0	203.0	228.0	246.0	263.0	274.0
Palisade (percentage of total thickness)	52.0	52.0	52.0	52.0	57.0	57.0	53.0	55.0
Date of planting seeds	Oct. 30, 1937				Feb. 24, 1938			
Date of harvesting plants	Dec. 29, 1937				April 20, 1938			

Statistical tests show that for stem heights and diameters, total dry weights, and leaf areas, the odds are at least 19:1 that nearly all the differences between the means of adjacent groups are significant. For both series, however, the odds are only about 4:1 that the difference between the water requirements of sets A and D is real. It should be mentioned in this connection that, for both series IV and V, the order of variation of water requirement with holard was the same as that of rate of transpiration per unit area. In series IV, both water requirement and rate of transpiration increased with greater holard, while in series V the opposite order held. A similar relation was noted in series I–III.

The diurnal variation of stomatal opening was determined on Dec. 3, 13, and 28 for series IV. On the first date, no difference in behavior of the stomata of the various groups was apparent, but on the other two days, the stomata of set D remained a little more widely open in the afternoon than those of the other three sets. For series V, a determination of stomatal behavior on April 13 disclosed no difference in the various sets; all remained open all day. In general, the behavior of the stomata appeared to be closely related to the transpiration rate.

At the close of the series, stomatal counts made from strips of epidermis from the lower surface of the fourth pair of leaves showed that the number per square millimeter increased rapidly with decreasing holard, set A having nearly twice as many as set D. The size of the stomata, both in guard cells and in length of opening, was in inverse proportion to the frequency, those in set A being only little more than half as large as those in D. Sections through the mesophyll of the same leaves showed a reduction in size of the mesophyll cells with reduced holard. The leaves were also thinner in the latter situations but the proportion of the mesophyll composed of palisade was unchanged. The reduced anatomy of the leaves of plants growing in soil with lower holards appeared to be largely a result of failure to expand under lessened turgor, rather than to a difference in the number of initial cells formed.

Summary

1. Plants of the Russian Mammoth variety of *Helianthus annuus* have been grown in containers holding about 130 pounds of dry soil under conditions of different holard, using two methods.

(a) One group of plants was grown in soil in which the holard was maintained near the field capacity by frequent additions of water; the other group was allowed to remove about two-thirds of the available soil moisture before the supply was replenished.

(b) Four groups of plants were grown in soil mixed at predetermined holards; no water was added at any time during the series. The hollards selected were approximately 11, 14, 17, and 20 per cent. of the dry

weight of the soil. The wilting coefficient of this soil was 7 per cent. and the field capacity 18 per cent.

2. In all series lower holard resulted in reduced growth in terms of stem diameter, leaf area, dry weight, and usually in stem height.

3. In experiments using method (a) above, water requirement and shoot-root ratio increased with greater available water, but in those using method (b) neither was altered appreciably.

4. Stomata were smaller and more numerous on plants with less available water. The leaves were also thinner, but the proportions of the leaf composed of palisade and sponge tissue were unchanged. The leaf anatomy appeared to be largely a case of failure of the cells to expand because of the reduced turgor, rather than to a difference in the number of initial cells formed.

5. Growth rate was affected by even small differences in holard, according to both methods used, and was affected by reduced holard long before any effect on stomatal opening or transpiration rate per unit area could be detected.

6. The rate of transpiration per unit of leaf surface was ordinarily affected when about two-thirds of the available soil moisture had been removed, the actual point depending upon the transpiration rate. This was true even when large plants with closely spaced roots were used, and the transpiration rate was between 1 and 2 grams per square decimeter per hour.

7. The stomatal opening appeared to be unaffected unless the holard was reduced to the point where the transpiration rate was lowered.

8. In all cases in which the transpiration rate and stomatal opening were affected, the leaves showed signs of wilting.

9. Rate of increase in leaf area fell off as the soil moisture was reduced, but after watering recovered to a value greater than that of the control plants. This effect was probably due largely to recovery of turgor; if at all ascribable to this cause, however, then more than 18 hours were required for complete recovery.

10. No increase in the rate of transpiration per unit area of leaf surface of plants recovered from wilting above that of control plants, such as reported by TUMANOW, could be detected.

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STORAGE OF ROOT RESERVES IN RHODES GRASS

H. WEINMANN

(WITH FIVE FIGURES)

Introduction

It is a well-known fact that the roots of many perennial herbaceous plants function as organs of storage, even where there are no special morphological modifications of the root system. Root-storage phenomena in herbaceous plants have been studied in detail by a number of workers. GRABER and associates (**7**) found that in lucerne, polysaccharides and nitrogen increased in percentage and amount in the roots during the mature stages whereas spring growth was associated with a decrease in the percentage of nitrogen and soluble sugars in both tops and roots. The authors conclude that "storage is greatly accelerated during the periods of blossoming and seed formation. At such periods, the plant produces reserve foods in excess of the amounts used in vegetative extension and a surplus is stored in the roots. . . ." WILLARD (**20**), likewise working on lucerne, made similar observations. ALDOUS (**1**) found that in the roots of *Vernonia baldwinii, Andropogon scoparius, Symphoricarpus vulgaris, Rhus glabra* and *Verbena stricta* the percentage of nitrogen, total sugars and starch in general decreased to about the time of flowering, after which considerable increases of these constituents were recorded. ALDOUS thinks that after the time of flowering "the plants begin to restore the reserves which must have been drawn on to produce the top growth." Similarly, GRANDFIELD (**8**) reports a 23.8 per cent. increase in the amount of total carbohydrates and a 16.8 per cent. increase in the amount of total nitrogen in the roots of *Verbena stricta* during the period between budding and maturity, and approximately the same increases in the roots of *Solidago rigida* and *Vernonia baldwinii*. According to BEWS and VANDERPLANK (**5**), the percentage of total carbohydrates increased during the entire season and that of total soluble sugars during winter only, in the corms of *Hypoxis rooperi*, while the percentages of the same compounds decreased in the leaves between spring and autumn. The authors think, however, that in this plant "the large carbohydrate reserves are drawn upon to only a very slight extent when new leaves are produced in spring."

More recently data have been collected with regard to the storage and utilization of root reserves in various grasses. STURKIE (**16**) found that the rootstocks of *Sorghum halepense* were developed after the maturing of aerial plant parts; this was confirmed by SAMPSON and MCCARTY (**14**) in *Stipa pulchra*, where an accumulation of carbohydrate reserves in the roots was observed during maturation. In the roots of *Digitaria eriantha* and *Themeda*

triandra, BEWS and BAYER (**4**) found only somewhat irregular fluctuations of sugars and polysaccharides, whereas in *Elyonurus ambiguus* and *Muehlenbergia gracilis*, MCCARTY (**9**) recorded an accumulation of sugars and starch in the stem bases, rhizomes and roots at the end of the season with the cessation of growth. That mineral elements may also be included in the substances stored in the roots, is shown by the work of RICHARDSON, TRUMBLE and SHAPTER (**12, 13**) on *Lolium subulatum* and *Phalaris tuberosa*. In both grasses, considerable losses of nitrogen, phosphorus, and potassium occurred from the herbage portions during maturity; amounting in *Phalaris tuberosa* to 36, 22, and 12 per cent. of the total intake of N, K_2O, and P_2O_5 respectively. In the case of *Phalaris tuberosa*, the losses of nitrogen and phosphorus could be accounted for by translocation to the basal internodes and to the root system; potassium was actually lost from all portions of the plant, amounting to 27.8 per cent. of the amount found in the entire plant shortly before flowering.

The writer, who has been engaged for several years in the study of the storage of root reserves in some South African Highveld grasses, found that in the roots of *Trachypogon plumosus*, *Tristachya hispida* and *Digitaria tricholaenoides*, the percentage of nitrogen and total sugars (**17**) as well as of phosphorus (unpublished data) decreased during the growing period and increased in autumn and winter. In connection with a study of the growth rhythm of the same three species and also of *Cynodon dactylon*, *Brachiaria serrata* and *Eragrostis chalcantha*, ALTONA (**2**) was recently able largely to confirm the results hitherto obtained by examining the percentages of nitrogen and total sugars in the roots of these grasses at their various stages of development. In general, the curves of nitrogen and total sugar content of the roots show a definite seasonal trend reaching a maximum in mid-winter and dropping at the time of shooting to reach a minimum at the time of flowering.

Seasonal chemical changes in the roots coincide largely with corresponding changes in the chemical composition of the herbage in the opposite direction. Decreases in the percentage of nitrogen, phosphorus, potassium and other constituents in the herbage during the growing season have been reported by many authors in various countries. When the total amounts of these constituents in the herbage per unit area are calculated, it is shown, however, that absorption continues for a considerable part of the growing season. While the decrease of the percentage of nitrogen, phosphorus and potassium during the greater part of the growing season is merely due to the progressive accumulation of carbohydrates, actual losses from the herbage of total amounts of these constituents occur during autumn when the percentages of the same constituents in the roots increase (**17**).

Naturally, mere percentage figures are not fully conclusive for the characterization of such processes as translocation and storage where various opposite processes or processes having opposite effects may go on at the same time. Thus, prolonged absorption of mineral elements by the roots from the soil may mask translocation of the same elements from shoots to roots, whereas an exceedingly high increase of one group of constituents may under certain circumstances result in a decrease of the percentage of another group, though that group may actually increase in amount. Only a determination of the total amounts of the various constituents present in shoots and roots at different stages of growth and maturation will indicate to what extent constituents have been translocated from shoot to root and vice versa. This, however, is rarely possible with plants growing in the field, since a collecting of the whole root bulk is mostly impracticable. For a more detailed investigation of the processes of absorption, translocation, and storage, pot experiments conducted under controlled conditions are more suitable.

The present contribution deals with the storage of root reserves in *Chloris gayana* KUNTH, Rhodes Grass, which was grown under controlled conditions in pots and was harvested at different stages of maturation.

Materials and methods

For this experiment cylindrical metal tins approximately 2 feet in height and with an open surface of 1250 cm^2 were used. These tins were first brought to equal weight by placing a few quartz stones in the bottom, and were then filled with ordinary veld soil collected at Frankenwald, the Botanical Research Station of the Witwatersrand University, near Johannesburg, South Africa, where the experiment was conducted.

The lower half of the tins was filled with 29.2 lb. of sub-soil consisting of coarse decomposed granite, the upper half with 32.2 lb. of top-soil (the figures expressing absolute dry weight). Top-soil as well as sub-soil were thoroughly mixed before being distributed into the single pots; the top-soil was also sieved through a wide-meshed sieve in order to exclude coarser stones. The top-soil used was an acid loamy sand, poor in organic matter and nutrients, particularly in nitrogen and phosphorus. The dry top-soil (as used) contained 29.85 per cent. stones (determined by using a sieve of 20 mesh-per-inch) and 2.35 per cent. organic matter (loss on ignition). The results of chemical and mechanical analyses of the top-soil fine earth are given in table I.

For the mechanical analysis the BOYOUCOS hydrometer method was used (**6**), nitrogen was determined by the ordinary Kjeldahl method, phosphorus and potassium were extracted by treating 100 gm. of air-dry soil with 200 ml. of 10 per cent. HCl on a boiling water bath for 3 hours. The maximum water retaining capacity of the top-soil (as used) was 34.0 per cent. and that

TABLE I

CHEMICAL AND MECHANICAL ANALYSES OF TOP-SOIL FINE EARTH. RESULTS GIVEN IN PERCENTAGE OF DRY MATTER

MECHANICAL ANALYSES			
PARTICLE SIZE IN MILLIMETERS			
SAND 1.0–0.05	SILT 0.05–0.005	CLAY < 0.005	TOTAL COLLOIDS < 0.008
% 76.5	% 8.1	% 15.4	% 16.0
CHEMICAL ANALYSES			
NITROGEN	PHOSPHORIC OXIDE	POTASH	
0.107	0.015	0.087	

of the sub-soil was 29.7 per cent., equalling approximately 20 lb. of water per pot.

Thirty six tins were filled with soil as described above; one series of 12 tins received no fertilizer treatment (0); another series of 12 tins received a fertilizer treatment in the form of sulphate of ammonia, phosphate, and potash salt (PNK); and the third series of 12 tins were given the same treatment as the second with the difference that a threefold quantity of ammonium sulphate was given (PN_3K). The actual amounts of nutrients applied per pot were as follows:

P = 6 gm. raw rock and superphosphate mixture (27 per cent.) = 1.44 gm. P_2O_5;

K = 1.2 gm. muriate of potash (60 per cent.) = 0.72 gm. K_2O;

N = 3 gm. sulphate of ammonia (21.1 per cent.) = 0.63 gm. N;

N_3 = 9 gm. " " " " = 1.89 gm. N.

The phosphate was distributed and mixed into the soil surface about one inch deep on Dec. 15, 1936; sulphate of ammonia and potassium chloride were applied in the form of a solution on Dec. 17; on the 18th a number of seeds of Rhodes Grass were sown into each pot. Germination was satisfactory and after about one month the seedlings were singled out so that finally ten plants remained in each pot.

In contrast to the plants of the PNK and PN_3K series the plants of the 0 series were very poor from the beginning, and it was feared that they would die on account of a marked deficiency of nutrients. In order to prevent this,

to ten pots of this series a small amount of PNK equalling 1/20 of the proper PNK treatment was given on January 25, 1937; two pots of this series were kept entirely unfertilized as an absolute control. The whole experiment thus consisted altogether of four treatments:

	0	pnk	PNK	PN_3K
Number of pots	2	10	12	12

It later became evident, however, that the plants of the 0 series overcame their first set back and did not die; on the other hand, the small pnk treatment was not able to produce any significant difference as compared with the 0 plants. Practically, both of these series can be regarded as control series; the plants of both series remained true "deficiency" plants.

The plants were kept in the open air but were, in the early stages of growth, sheltered against heavy storms by means of a movable roof of corrugated iron which was removed as soon as rain had ceased. For watering, collected rain water was used and the water content of the pots was kept more or less constant by frequent weighing. The water supply was controlled according to the requirements of the growing plants. Thus, when the seeds were sown into the pots the soil possessed a water content equalling 33 per cent. of the maximum water retaining capacity of the pots; the water content was raised to 50 per cent. of the M.W.R.C. on Dec. 30, to 60 per cent. on Feb. 13, to 70 per cent. on March 2, and to 75 per cent. on April 3. On April 17, when the plants approached maturity, all watering was stopped and the pots were allowed to dry out gradually.

The plants were harvested at three different stages of maturity; namely, three to four pots of each series on March 23, April 17, and May 18, 1937. The two pots of the absolute control series (0) were harvested on the last date as well. The total herbage of each pot was first cut off directly at the base including also the subaerial parts of the stems, and the whole root system was collected by washing it free from the surrounding soil. Care was taken to avoid losses of roots, and at the same time to shorten the procedure of washing as much as possible, in order to avoid any risk of loss of constituents from the tissue by leaching. Roots and shoots were then air dried in a glass house. When air-dry, their weight was determined; hereafter the material of one treatment and harvest—roots and shoots separately—was united and finely ground. The ground material was stored in airtight bottles and kept for the various determinations.

The following constituents were determined in shoots and roots: Total sugars, total hydrolyzable carbohydrates, nitrogen, phosphorus, potassium and soluble ash.

Total sugars.—5 gm. of the airdry substance were (after the addition of 1 gm. $CaCO_3$) extracted twice with 200 ml. of 95 per cent. alcohol for a

period of two hours under a reflux condenser. The residue was then removed by filtering and the alcohol distilled off under reduced pressure; the remaining syrup was taken up with distilled water, cleared with neutral lead acetate, and deleaded with potassium oxalate. The extract was then hydrolyzed by boiling with HCl under a reflux condenser for 1½ hours (2.5 ml. conc. HCl were added to 100 ml. solution). Hereafter the solution was neutralized and made up to 500 ml. volume. The reducing power of this extract was then estimated by the MUNSON and WALKER method (**10**) followed by the BERTRAND method for the estimation of cuprous oxide (**3**). From the amount of copper precipitate the glucose equivalent was found by reference to the tables of MUNSON and WALKER.

Total hydrolyzable carbohydrates.—As recommended by BEWS and VANDERPLANK (**5**), 5 gm. of airdry substance were hydrolyzed with 200 ml. of 1.1 per cent. HCl in a beaker, covered with a watch glass, on a boiling water bath for 4½ hours. The hydrolyzed and filtered extract was then neutralized, cleared and deleaded, made up to 500 ml. volume, and its reducing power determined as described above. The results were also expressed as glucose.

Nitrogen.—Nitrogen was estimated by the ordinary Kjeldahl method, using a mixture of K_2SO_4 and $CuSO_4$ (9:1 parts by weight) as a catalyst in the digestion.

Phosphorus.—A sample of the plant material was incinerated after the addition of saturated lime water in order to avoid losses of organic phosphorus in the ignition. After removal of the silica, phosphates were precipitated in the nitric acid extract of the plant ash as ammonium phosphomolybdate according to LORENZ (**18**).

Potassium.—Potassium was determined as potassium perchlorate in the HCl extract of the plant ash after removal of silica, sulphates, phosphates, calcium, iron, aluminium, and magnesium (**18**).

Soluble ash.—A weighed ash sample of the plant material was treated with hot HCl. The liquid was then filtered off and the remaining insoluble residue (SiO_2) was washed, ignited, and weighed. The difference total plant ash minus SiO_2 was reported as soluble ash.

Dry matter.—The dry matter content was determined by drying a separate sample in an electric oven for 12 hours at a temperature of 105° C. All results of the chemical determinations were expressed as percentages of dry matter.

Results

YIELDS AND GENERAL DEVELOPMENT OF THE PLANTS

In their general development and growth rhythm, the plants of the PNK and PN_3K series were soon far in advance of the plants of the 0 and pnk series. The leaves of the PN_3K plants had a darker green color than the

leaves of the other plants, owing to their high nitrogen supply; the plants of 0 and pnk series, on the other hand, were poor and yellowish, and were not able to form stems and inflorescences. Both, PNK and PN_3K plants, produced stems and inflorescences, but the PN_3K plants did not only form more bulk than the PNK plants but produced also a larger number of inflorescences per plant (see fig. 1). The final average number of inflorescences formed by

FIG. 1. Inflorescences produced under varying nutrients. From left to right:
pnk series: received 1/20 of proper PNK treatment;
PNK series: received proper PNK treatment;
PN_3K series: three-fold quantity of ammonium sulphate.
(Photographed April 17, 1937).

ten plants (as counted on April 17 after the flowering period was finished) was 10.5 and 27.2 for PNK and PN_3K plants respectively. Maturation was accelerated by both these fertilizer treatments: the plants of PNK and PN_3K treatment possessed relatively early (middle of March) a fairly large proportion of dead, yellowish leaves—PN_3K plants again more than PNK plants—whereas 0 and pnk plants remained in the green, vegetative stage throughout the growing season, though yellowing occurred here to a certain extent, probably owing to deficiency of nutrients.

Three to four pots of each series were harvested at three different stages of maturation, namely on March 23, April 17, and May 18, 1937, respectively. At the first date, flowering was just commencing, while at the second date the flowering period was definitely finished. The first frosts were recorded on April 22, 24, and 29, followed by frosts on every day during May with the exception of a warm spell from the 6th to the 14th. The third harvesting date (May 18), was, therefore, well after the growing season had

ceased. At this last date, the PN_3K plants possessed only very few green leaves, the PNK plants a few more, whereas the plants of 0 and pnk series were still almost entirely green. While it is frequently observed that nitrogen in excess retards maturation, the earlier maturation of the PN_3K plants in this case may be partly due to the greater daily fluctuations in the water content of the pots of these better developed plants owing to their higher total transpiration. Seeding was in full swing near the last harvesting date and many seeds had fallen before this day. Further losses of seeds in handling the plants were unavoidable to a certain extent but, as far as possible, seeds were collected and included in the samples.

The results of the yield determinations—expressed as grams dry weight of shoots, roots, and total plant matter per pot (ten plants)—are given in table II and represented in figure 2. The figures are averages of all three or

TABLE II

YIELD OF PLANTS GROWN UNDER VARIOUS NUTRIENT CONDITIONS. GRAMS OF DRY MATTER PER TEN PLANTS

DATE	0	pnk	PNK	PN_3K
		SHOOTS		
	gm.	*gm.*	*gm.*	*gm.*
March 23, 1937	.....	8.6 ± 0.5	78.7 ± 2.9	121.1 ± 1.3
April 17, 1937	.....	14.0 ± 0.7	83.8 ± 8.2	136.3 ± 6.3
May 18, 1937	15.5 ± 1.4	16.3 ± 0.7	87.3 ± 3.9	130.0 ± 3.5
		ROOTS		
March 23, 1937	.....	13.9 ± 1.4	51.4 ± 2.5	71.3 ± 3.2
May 17, 1937	.....	25.8 ± 4.2	62.6 ± 2.6	74.3 ± 3.0
May 18, 1937	19.9 ± 0.6	21.2 ± 1.0	77.3 ± 4.0	92.4 ± 4.8
		TOTAL		
March 23, 1937	.....	22.5 ± 1.5	130.1 ± 3.8	192.4 ± 3.5
April 17, 1937	.....	39.8 ± 4.3	146.4 ± 8.6	210.6 ± 7.0
May 18, 1937	35.4 ± 1.5	37.5 ± 1.2	164.6 ± 5.6	222.4 ± 6.0

four pots harvested at each date and are given together with their corresponding standard errors. The standard error (s) was calculated by means of the formula:

$$s = \pm \sqrt{\frac{n(n-1)}{\Sigma v^2}},$$

where Σv^2 = sums of the squares of the deviations of the single observations from the mean, and n = number of single observations.

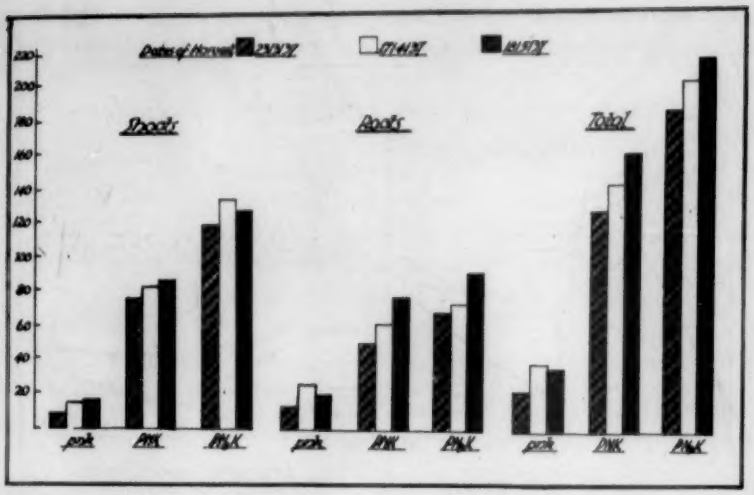

FIG. 2. Yields; grams of dry matter per ten plants.

A comparison of the yields of the three sampling dates shows that with regard to the shoots, growth was practically finished on April 17, no significant difference being between the yields of April 17, 1937 and May 18, 1937 with the possible exception of the pnk plants which show also during this period a slight and perhaps significant increase of 2.3 gm. $\pm$ 0.99. The growth rhythm was, however, different in the *roots*—at least in PNK and PN_3K plants—which also increased in the very last part of the growing season, *i.e.*, after flowering and during formation of seeds. The changes in the root system between April 17 and May 18 were as follows:

in pnk a decrease of 4.6 gm. $\pm$ 4.3 (= – 17.8 per cent.);
in PNK an increase of 14.7 gm. $\pm$ 4.8 (= + 23.5 per cent.);
in PN_3K an increase of 18.1 gm. $\pm$ 5.7 (= + 24.4 per cent.).

The decrease is small and uncertain for its relatively high error in the pnk plants. At any rate, the pnk plants were, on account of a general deficiency of nutrients, hindered in their normal development. But significant increases of the root system occurred in the well-fertilized plants during the late stages of maturation, when aerial growth had already ceased. This is in agreement with observations of several other workers already mentioned.

CHEMICAL CHANGES IN THE PLANTS DURING MATURATION

The results of the chemical determinations are given in table III and figure 3. The figures show that in the shoots the percentage of most constituents determined decreased during maturation. This is particularly distinct with regard to sugars, hydrolyzable carbohydrates, phosphorus and potassium[1];

[1] Potassium and soluble ash could not be determined in the pnk series owing to lack of sufficient material.

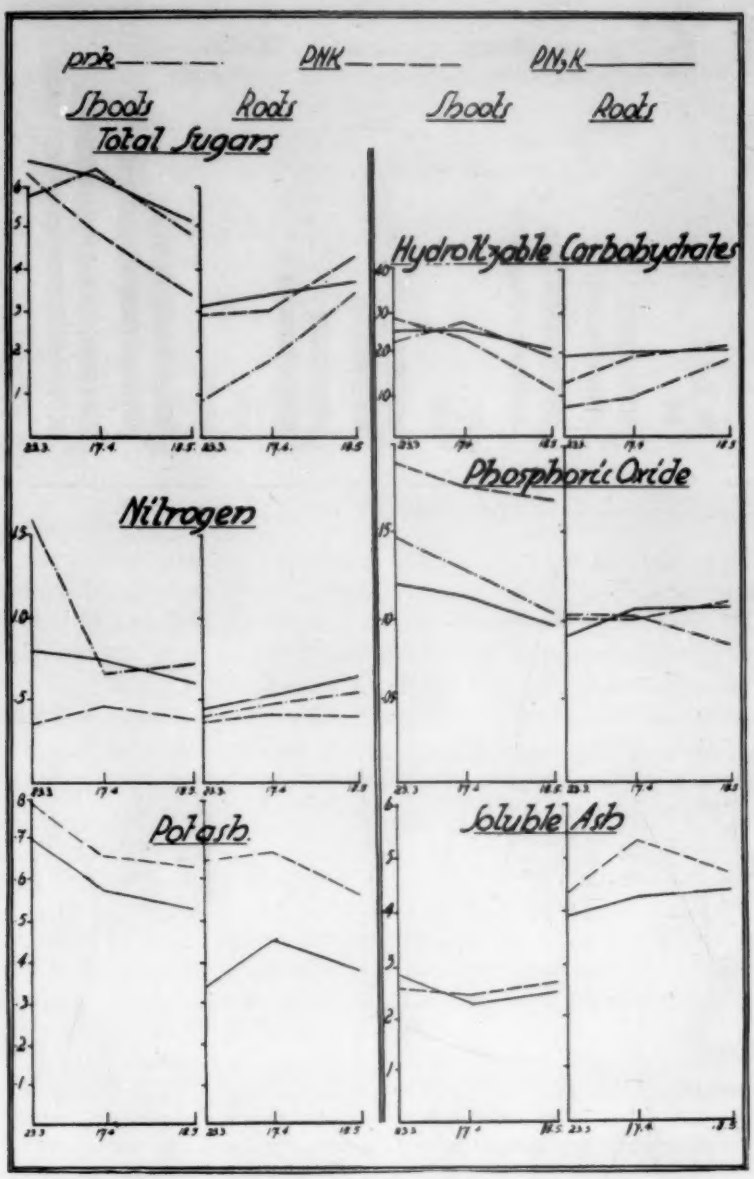

FIG. 3. Chemical composition of shoots and roots; percentages of dry matter.

percentage nitrogen decreased distinctly at least in the PN_3K series, while the percentage of soluble ash[1] changed only little. In the roots, however, the percentages of most constituents determined increased during maturation,

TABLE III

CHEMICAL COMPOSITION OF SHOOTS AND ROOTS.
EXPRESSED AS PERCENTAGE OF DRY MATTER

CONSTITUENT	DATE	pnk		PNK		PN_3K	
		SHOOTS	ROOTS	SHOOTS	ROOTS	SHOOTS	ROOTS
		%	%	%	%	%	%
Total Sugars	3/23/37	5.80	0.97	6.52	2.90	6.55	3.04
	4/17/37	6.34	1.87	4.72	2.97	6.25	3.31
	5/18/37	4.77	3.38	3.32	4.37	5.06	3.73
Hydrolyz. Carbohydrates	3/23/37	23.25	8.06	29.50	14.00	25.50	20.65
	4/17/37	27.20	10.17	23.75	20.27	26.50	21.00
	5/18/37	20.75	19.20	11.95	21.90	21.25	21.75
Nitrogen	3/23/37	1.55	0.41	0.35	0.36	0.81	0.47
	4/17/37	0.65	0.48	0.48	0.40	0.72	0.52
	5/18/37	0.73	0.53	0.37	0.39	0.59	0.62
Phosphoric Oxide	3/23/37	0.149	0.104	0.192	0.096	0.122	0.087
	4/17/37	0.127	0.097	0.178	0.096	0.113	0.099
	5/18/37	0.097	0.083	0.167	0.108	0.093	0.105
Potash	3/23/37	.....	.....	0.77	0.63	0.69	0.33
	4/17/37	.....	.....	0.65	0.64	0.56	0.44
	5/18/37	.....	.....	0.62	0.55	0.52	0.36
Soluble Ash	3/23/37	.....	.....	2.52	4.28	2.74	3.90
	4/17/37	.....	.....	2.38	5.27	2.20	4.19
	5/18/37	.....	.....	2.53	4.69	2.45	4.35

though in some cases the changes were considerably smaller than in the shoots. Thus, in the roots of the PN_3K plants the percentages of sugars and hydrolyzable carbohydrates increased only slightly. The same holds true for nitrogen in all series and for phosphorus in the PNK and PN_3K series, while the percentage of potassium decreased in the roots of both well fertilized series towards the end of maturation and that of soluble ash in the PNK series only.

From the percentage figures and the dry weight of shoots and roots, the actual amounts of constituents in shoots and roots of each treatment and date were calculated. The results are given in table IV, expressed as grams of constituents per ten plants and represented in figure 4. As indicated above, the calculation of total amounts offers a much better means of deciding whether a constituent has actually decreased or increased in a portion of a plant, since a change in the percentage of a constituent does not necessarily mean that a corresponding change of the actual amount of the constituent in the plant or plant part has occurred.

With the exception of soluble ash, which remained more or less constant over the whole period of maturation, all constituents decreased distinctly in

TABLE IV

ACTUAL AMOUNTS OF CONSTITUENTS IN SHOOTS AND ROOTS.
EXPRESSED AS GRAMS PER TEN PLANTS

CONSTITUENT	DATE	pnk			PNK			PN_3K		
		SHOOTS	ROOTS	TOTAL	SHOOTS	ROOTS	TOTAL	SHOOTS	ROOTS	TOTAL
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Total sugars	3/23/37	0.50	0.13	0.63	5.13	1.49	6.62	7.95	2.17	10.12
	4/17/37	0.89	0.48	1.37	3.96	1.86	5.82	8.53	2.46	10.99
	5/18/37	0.78	0.71	1.49	2.88	3.38	6.26	6.57	3.45	10.02
Hydrolyz. carbohydrates	3/23/37	2.0	1.1	3.1	23.2	7.2	30.4	30.9	14.7	45.6
	4/17/37	3.8	2.6	6.4	19.9	12.7	32.6	36.2	15.6	51.8
	5/18/37	3.4	4.1	7.5	10.4	16.9	27.3	27.6	20.1	47.7
Nitrogen	3/23/37	0.13	0.06	0.19	0.28	0.16	0.44	0.98	0.33	1.31
	4/17/37	0.09	0.12	0.21	0.40	0.25	0.65	0.98	0.39	1.37
	5/18/37	0.12	0.11	0.23	0.32	0.30	0.62	0.77	0.57	1.34
Phosphoric oxide	3/23/37	0.013	0.014	0.027	0.151	0.049	0.200	0.148	0.062	0.210
	4/17/37	0.018	0.025	0.043	0.149	0.060	0.209	0.154	0.074	0.228
	5/18/37	0.016	0.018	0.034	0.146	0.084	0.230	0.121	0.097	0.218
Potash	3/23/37	.....	.....	.....	0.61	0.32	0.93	0.84	0.24	1.08
	4/17/37	.....	.....	.....	0.54	0.40	0.94	0.76	0.33	1.09
	5/18/37	.....	.....	.....	0.54	0.43	0.97	0.68	0.33	1.01
Soluble ash	3/23/37	.....	.....	.....	1.98	2.20	4.18	3.32	2.78	6.10
	4/17/37	.....	.....	.....	1.99	3.30	5.29	3.00	3.12	6.12
	5/18/37	.....	.....	.....	2.21	3.63	5.84	3.18	4.02	7.20

actual amount in the shoots of the well fertilized and normally developed plants of the PNK and PN_3K series, particularly during the last part of maturation. In the roots, on the other hand, all constituents increased in total amount—in most cases right through the period of maturation. Some exceptions are found in the pnk plants which may be explained by their abnormal development owing to a general deficiency; as mentioned above, these plants never reached the stage of physiological maturation.

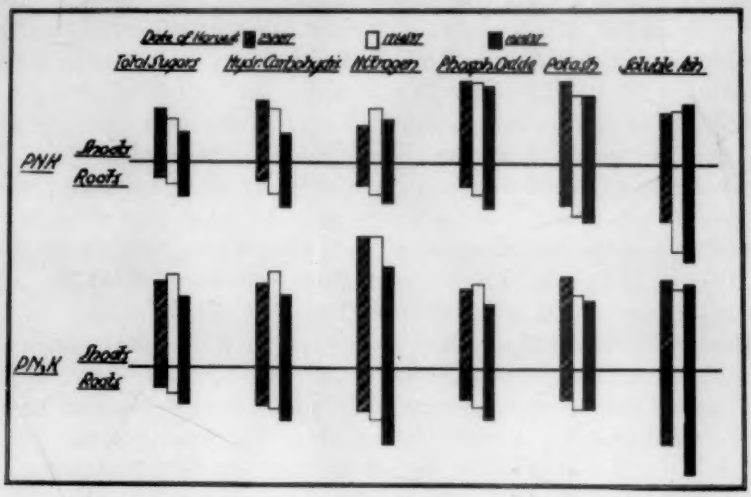

FIG. 4. Actual amounts of constituents (different scales).

If the total amounts of nitrogen and mineral elements present in the entire plant at different stages of maturation are compared with each other, it is revealed that absorption must have continued at least for a part of the maturation period. The differences in physiological maturation between PNK and PN_3K plants are well reflected in their total uptake of nitrogen and mineral elements. Nitrogen absorption was practically completed by March 23 in the PN_3K series, whereas in the less advanced PNK plants considerable absorption of nitrogen took place until April 17; in the immature remaining pnk plants nitrogen absorption continued until May 18. Similarly, absorption of phosphorus continued in the PNK series up to the last sampling date on May 18, but had already finished in the PN_3K series on April 17. Potassium increased very slightly during maturation in the PNK plants, but no absorption of potassium occurred during the same period in the more advanced PN_3K plants. In the latter series, there was even a loss of total amount of potassium during the last part of maturation, amounting to 0.08 gm. K_2O per ten plants. Though this small loss cannot be regarded as significant, since the variations of the total dry matter between pots of the same harvest would account for an error of $\pm$ 0.045, it should be remembered again

that substantial losses of potash from all portions of the plant during the late stages of maturation have been recorded by RICHARDSON, TRUMBLE and SHAPTER (13) in *Phalaris tuberosa*. Similar losses of nitrogen and mineral elements by remigration into the soil during maturation are also reported by WILFARTH, RÖMER and WIMMER (19), SEKERA (15) and PENSTON (11).

The figures of soluble ash are only of limited physiological value, since some mineral elements (such as phosphorus and sulphur) are partly lost in the ignition and are therefore not quantitatively included. It would appear, however, from the total amounts of soluble ash that absorption continued right through the period of maturation, again more pronounced in the PNK series than in the PN_3K series. In both series, the bulk of mineral elements absorbed during this period accumulated only in the roots, not appreciably affecting percentages and amounts of soluble ash in the shoots.

It should be remembered that in the interpretation of the data presented a number of actual and possible errors must be taken into account. The standard error of the dry matter (as given in table II) would naturally appear proportionally as standard error of each single constituent of the dry matter, limiting the conclusions as to the significance of the differences recorded. Possible variations in the chemical composition of plants of the same treatment and harvest but from different pots are not even included here, since only combined samples were analyzed. In addition, some inherent errors in methods would probably influence the results at least slightly: small amounts of plant matter were lost at the end of maturation by the loss of seeds and rain may have leached out constituents from dead leaves.

Furthermore, as far as carbohydrates are concerned, one would not expect that the constituents disappearing from the shoots should be recovered in exactly the same amounts in the roots. Sugars translocated from shoots to roots may there be polymerized to various types of polysaccharides, part of which (such as pentosans, hemi-celluloses and celluloses) do not appear as "hydrolyzable carbohydrates" under the conditions of hydrolysis employed. On the other hand, photosynthetic activity followed by immediate translocation of the photosynthetic products to the roots, would result in an increase of the amounts of carbohydrates in the roots without a corresponding decrease in the shoots. Appreciable losses of carbohydrates may possibly occur in the respiration of living plant parts, particularly also of actively growing roots, or in the decomposition of dead parts.

While for all these reasons too far-reaching conclusions should be avoided, the results should be conclusive proof that in *Chloris gayana* during the period of maturation appreciable amounts of organic reserves as well as of mineral elements are translocated from shoots to roots, in part accompanied by continued contemporaneous absorption by the roots of nitrogen and mineral nutrients from the soil.

The continuous accumulation of dry matter and constituents effected in this way, in the root portion of the plants after flowering, becomes most evident when the proportions of root dry matter and of root constituents are

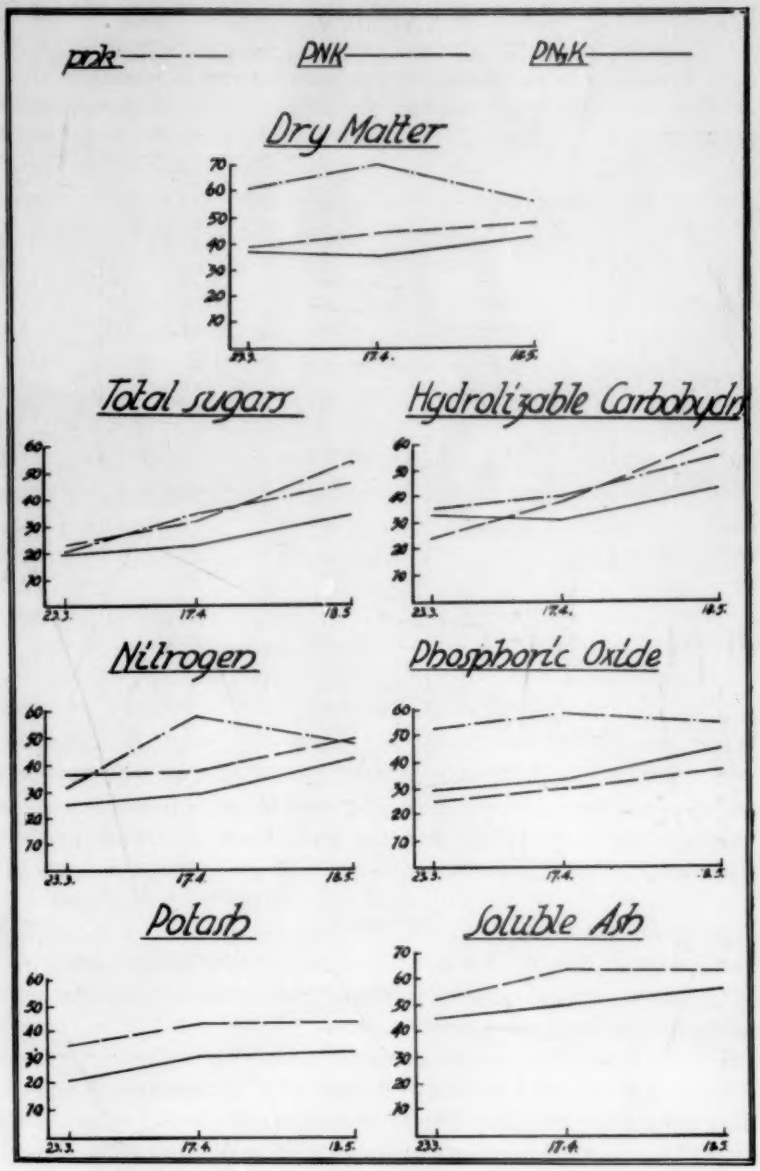

FIG. 5. Relative amounts of dry matter and constituents in the roots; percentages of the total amount in the plant.

expressed as percentages of the corresponding amounts present in the entire plant $\left(\frac{\text{amount in roots} \times 100}{\text{amount in whole plant}}\right)$. As can be seen from table V and figure 5,

TABLE V

RELATIVE AMOUNTS OF DRY MATTER AND CONSTITUENTS IN THE ROOTS. EXPRESSED AS PERCENTAGES OF THE TOTAL AMOUNT IN THE PLANT

CONSTITUENT	DATE	pnk	PNK	PN_3K
		%	%	%
Dry matter	3/23/37	61.8	39.6	37.1
	4/17/37	64.8	42.8	35.3
	5/18/37	56.6	47.0	41.5
Total sugars	3/23/37	20.7	22.5	19.8
	4/17/37	35.0	32.0	22.4
	5/18/37	47.7	54.0	34.4
Hydrolyz. carbohydrates	3/23/37	35.5	23.7	32.2
	4/17/37	40.7	38.9	30.1
	5/18/37	54.7	61.9	42.2
Nitrogen	3/23/37	31.6	36.4	25.2
	4/17/37	57.2	38.5	28.5
	5/18/37	47.8	48.4	42.5
Phosphoric oxide	3/23/37	51.9	24.5	29.5
	4/17/37	58.2	28.7	32.5
	5/18/37	52.9	36.5	44.5
Potash	3/23/37	.....	34.4	22.2
	4/17/37	.....	42.6	30.3
	5/18/37	.....	44.3	32.7
Soluble ash	3/23/37	.....	52.6	45.6
	4/17/37	.....	62.4	51.0
	5/18/37	.....	62.2	55.8

these relative amounts of dry matter and constituents in the roots increased distinctly during maturation, the only exceptions occurring in the pnk series, probably owing to the retarded development of these deficiency plants.

Summary

Chloris gayana KUNTH, Rhodes Grass, was grown under controlled conditions in pots receiving different fertilizer treatments and was harvested at three different stages of maturity.

In the well fertilized plants significant increases of the root system occurred after the time of flowering and during the formation of seeds, when growth of the aerial parts had already ceased.

During this period considerable amounts of sugars, hydrolyzable carbohydrates, nitrogen, phosphorus and potassium were lost from the shoots and were largely recovered in the roots.

In addition to translocation from the shoots, nitrogen and mineral elements also accumulated in the roots to a certain extent by simultaneous absorption from the soil. The duration of the absorption period varied, however, for different elements; it also varied for plants grown under different nutritional conditions and hence reaching different stages of physiological maturation.

The relative amounts of dry matter and constituents present in the roots (expressed as percentages of the total amounts present in the whole plant) increased, in most cases, considerably during autumn, indicating a continuous storage of these substances in the roots with the approach of maturity.

Abnormally developed and immature remaining deficiency plants showed in part a correspondingly abnormal behavior with regard to root storage and absorption.

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THE MINERAL COMPOSITION OF THE ALCOHOLIC EXTRACT OF POTATO LEAVES AND ITS RELATIONSHIP TO CROP YIELDS

C. E. Beauchamp

(WITH THREE FIGURES)

Introduction

In previous papers on the nutrition of the sugar-cane plant (**3, 4**), it was shown that the mineral composition of the alcoholic extracts of cane leaves taken at an early stage of growth, from plots having different fertilizer treatments, bore a direct relationship to growth and final yield. Growth was measured in terms of average stalk length, average number of stalks per stool, and total cane weight. In that investigation it was shown that the composition of the skeleton of the leaves deprived of the minerals soluble in alcohol, had no correlation at all with cane growth. These results were checked in another fertilizer experiment (**2**) conducted in an adjacent field consisting of the same soil type and using the same cane variety. The interesting results obtained in these investigations prompted the writer to study the mineral composition of alcoholic extracts of potato leaves taken from a potato fertilizer experiment.

The experiment was started on February 8, 1936, on a Matanzas red clay. This soil type is very deep and friable, the difference between soil and sub-soil being scarcely noticeable. Its pH was about 6.5. The potato variety planted was Irish Cobbler. Plots of 1/47 acre were 99 by 9.30 feet. Each plot comprised five rows 1.86 feet apart.

The fertilizer was applied at planting time at the rate of 1500 lb. per acre. Eight different treatments were compared including checks, each one being replicated three times at random.

The fertilizer treatments compared were as follows:

No. 1—Check	No. 5— 8–10–15
No. 2—6– 8–10	No. 6—10– 8– 0
No. 3—8–10–10	No. 7—10– 8–10
No. 4—8–10– 0	No. 8—10– 8–15

The fertilizer mixtures were prepared as shown in table I.

On March 30, 1936, fifty-two days after planting, leaf samples were taken from each plot. Leaves from similar treatments were mixed. Aliquots, enough to fill a one-quart, wide-mouthed bottle, were used. 95 per cent. alcohol was poured in so as to cover the leaf material. All bottles were then pasteurized at 60° C. for 20 min. and sealed. The green matter of the leaves was removed using Soxhlet extractors. The extraction was

TABLE I

FERTILIZER MIXTURES USED IN GROWTH OF IRISH COBBLER POTATOES

FORMULA	POUNDS PER ACRE	PLANT FOOD			UREA	NITRATE OF SODA	SULPHATE OF AMMONIA	DOUBLE SUPERPHOSPHATE	SULPHATE OF POTASH	MURIATE OF POTASH
		NH_3	P_2O_5	K_2O						
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
6–8–10	1500	90	120	150	27	160	180	267	156	123
8–10–10	1500	120	150	150	27	160	300	333	156	123
8–10–0	1500	120	150	.....	27	160	300	333	.....	.....
8–10–15	1500	120	150	225	27	160	300	333	234	184
10–8–0	1500	150	120	.....	27	160	420	267	.....	.....
10–8–10	1500	150	120	150	27	160	420	267	156	123
10–8–15	1500	150	120	225	27	160	420	267	234	184

discontinued when the alcoholic leachings came out colorless. The quantity of leaf material taken from each treatment was arbitrary. The amount of alcohol used for each sample was also variable depending upon the volume necessary to effect a complete extraction. The alcoholic extracts were then transferred to beakers and evaporated on the water bath. The residues from the alcoholic extracts as well as the leaf skeletons were dried to constant weight. The former were digested with sulphuric acid until colorless. The volume was then made up to 500 ml. and kept in sealed bottles until analyzed.

Methods

NITROGEN

Twenty-five ml. aliquots of the solution were digested for 2 hours with 5 ml. of H_2SO_4 plus 2 gm. of K_2SO_4 and 0.02 gm. $CuSO_4$ in a 250-ml. Kjeldahl flask.

The samples were transferred to the VanSlyke-Cullen aeration apparatus, and about 18 ml. of saturated NaOH were added. Aeration was continued for four hours into 200 ml. of standard H_2SO_4 (0.01990 N). Back titration was made with NaOH of the same normality.

PHOSPHORUS

The procedure as outlined in the methods of the Association of Official Agricultural Chemists (1935) was used on 50-ml. aliquots of the solution. Titration was made with 0.05140 N NaOH and 0.02570 N HNO_3.

POTASSIUM

The 25-ml. aliquots were evaporated to dryness and heated to remove H_2SO_4. Dehydration, to remove SiO_2, was made by adding 4–5 ml. of con-

centrated HCl, evaporating to dryness on the steam bath, heating for one hour and taking up the residue in hot H_2O. The dehydrated SiO_2 was removed by filtration, and calcium was precipitated as the oxalate. The solutions were again filtered, evaporated to dryness, heated to expel ammonium salts, and K_2O determined by the chloroplatinic method as described in the A.O.A.C., 1935.

Calcium

62.5-ml. aliquots were evaporated and heated to remove H_2SO_4. The residues were taken up in H_2O, and calcium was precipitated as described in A.O.A.C., 1935, by adding 5 ml. of saturated $(NH_4)_2C_2O_4$.

The CaC_2O_4 was removed by filtration through an asbestos prepared Gooch. After dissolving the precipitate in H_2SO_4 (1:2), titration was made with $KMnO_4$. This was very weak and was made up twice, with normalities of 0.011517 and 0.01161.

Magnesium

Magnesium was determined in the filtrate from calcium by precipitation with 8-hydroxyquinoline. The solutions were made to about 150 ml., adjusted to neutrality, and heated to 70–80° C. Four ml. of 5 per cent. 8-hydroxyquinoline in 2 N acetic acid were added. Two ml. in excess of 1:2 NH_4OH were added, and the solutions were allowed to stand for one hour until the precipitate had settled. Filtration was made through sintered glass crucibles, and the precipitate was washed well with cold H_2O.

The crucibles containing the precipitate were dried to constant weight at 105° C. The percentage of magnesium was calculated from the weight of $Mg(C_9H_6NO)_2 \cdot 2H_2O$ obtained.

Harvesting

On April 17, 1936, the potatoes were dug. The yield from each plot was carefully weighed and classified into no. 1's and no. 2's according to size.

Results

Table III shows the yield response of the potatoes to the different fertilizer treatments applied. The yield of the check plots was insignificant. All fertilizer mixtures resulted in very high increases when compared with check plots. Among the fertilized plots, the differences in yield were not marked, yet they were sufficient to show how the crop responded to the various fertilizer treatments.

The increments of potash in the fertilizers were followed by a marked yield increase as shown by comparing yields of treatments 8–10–0, 8–10–10, 8–10–15 and 10–8–0, 10–8–10, and 10–8–15. In the first three treatments

TABLE II

YIELD OF "NUMBER 1" AND "NUMBER 2" POTATOES GROWN IN EXPERIMENTAL PLOTS

	Treatments	Plot number	Yield per plot		Total	No. 1	No. 2
			No. 1	No. 2			
			lb.	*lb.*	*lb.*	%	%
1.	Check	5	8	38	46	17.0	83.0
		17	4	24	28	14.0	86.0
	Average		6	31	37	15.5	84.5
2.	6–8–10	3	280	22	242	90.9	9.1
		7	190	16	206	92.2	7.8
	Average		205	19	224	91.6	8.4
3.	8–10–10	9	209	18	227	92.0	8.0
		11	190	24	214	88.8	11.2
	Average		199.5	21	220.5	90.4	9.6
4.	8–10–0	1	177	28	205	86.3	13.7
		8	114	18	132	86.3	13.7
		12	129	16	145	98.0	11.0
	Average		140	21	161	87.2	12.8
5.	8–10–15	2	246	21	267	92.0	8.0
		6	235	21	256	92.0	8.0
		18	182	15	197	92.4	7.6
	Average		221	19	240	92.1	7.9
6.	10–8–0	13	153	16	169	90.5	9.5
		15	164	16	180	91.1	8.9
		19	141	17	158	89.2	10.8
	Average		152.7	16.3	169	90.3	9.7
7.	10–8–10	10	221	11	232	95.3	4.7
		14	205	14	219	93.7	6.3
	Average		213	12.5	225.5	94.5	5.5
8.	10–8–15	4	274	20	294	93.3	6.7
		16	224	16	240	93.3	6.7
		20	222	15	237	93.7	6.3
	Average		240.0	17.0	257.0	93.5	6.5

the yields were 79.43, 105.98 and 120.79 cwt. per acre respectively. In table II, it is seen that the percentage of no. 1 potatoes increased slightly but consistently with increments of potash in the fertilizers.

Increments of nitrogen in this experiment did not increase yields. With 10 per cent. of potash and 8 per cent. of phosphoric acid an increase of nitrogen from 6 to 10 per cent. NH_3, did not have any effect at all on the

TABLE III

AVERAGE YIELDS PER ACRE

TREATMENTS	WEIGHTS PER ACRE		
	No. 1	No. 2	TOTAL
	cwt.	*cwt.*	*cwt.*
1. Check	2.82	14.57	17.39
2. 6–8–10	96.35	8.93	105.28
3. 8–10–10	93.76	9.87	103.63
4. 8–10–0	65.80	9.87	75.67
5. 8–10–15	103.87	8.93	112.80
6. 10–8–0	71.77	7.66	79.43
7. 10–8–10	100.11	5.87	105.98
8. 10–8–15	112.80	7.99	120.79

yields. This is brought out in table III by comparing yields of plots treated with mixtures 6–8–10 and 10–8–10.

In comparing treatments with 8 and 10 per cent. of ammonia it is unfortunate that the content of phosphoric acid varies; 10 per cent. in the first case and 8 per cent. in the second. In spite of this irregularity there is practically no significant difference between the yields of treatments 8–10–0 and 10–8–0, being 75.67 and 79.43 cwt. per acre, respectively. The same holds in comparing treatments 8–10–10 and 10–8–10; the respective yields being 103.63 and 105.98 cwt. per acre. There appears to be a slight increase in yield as a result of a nitrogen increment in treatment 10–8–15 as compared with 8–10–15. In these cases the yields were 120.79 and 112.80 cwt. per acre, respectively. This difference, however, did not prove to be significant when subjected to statistical analysis.

No comparison can be made between phosphorus increments in this experiment because both phosphorus and nitrogen vary at the same time. It was shown above, however, that increasing the nitrogen did not result in yield increases. In the case of phosphorus, table III shows that an increase of this nutrient had a negative effect on the yield. This is brought out by comparing yields from treatments 10–8–10 *vs.* 8–10–10 and 10–8–15 *vs.* 8–10–15. From these comparisons it cannot be definitely decided whether phosphorus increments depressed yields as a result of a low nitrogen content in the fertilizer or if the nitrogen increments were ineffective due to a lower phosphorus content.

The analyses of the alcoholic extracts of the leaves of the various treatments appear in table IV.

The figures in this table are very significant. If the yields per acre are compared with the total percentage of nutrients in the dry matter of the leaf extracts a very good correlation is observed. This correlation is readily seen in figure 1. The lowest yield obtained in the check plots and in

TABLE IV

COMPOSITION OF THE LEAF EXTRACTS IN PERCENTAGE OF DRY EXTRACT. AVERAGE OF TWO DETERMINATIONS

TREATMENTS	YIELD PER ACRE	K_2O	P_2O_5	N	CA	MG	TOTAL
	cwt.	%	%	%	%	%	%
1. Check	17.39	2.87	0.155	0.429	0.079	0.115	3.648
2. 6–8–10	105.28	9.77	0.460	0.974	0.095	0.337	11.638
3. 8–10–10	103.63	10.76	0.534	1.042	0.084	0.432	12.852
4. 8–10–0	75.67	3.97	0.233	1.034	0.126	0.427	5.790
5. 8–10–15	112.80	11.29	0.509	1.027	0.084	0.321	13.231
6. 10–8–0	79.43	5.84	0.237	1.306	0.106	0.654	8.143
7. 10–8–10	105.98	11.27	0.515	1.050	0.095	0.507	13.437
8. 10–8–15	120.79	9.97	0.568	1.208	0.079	0.390	12.215

turn, the leaf extracts from these plots, showed the lowest percentage of total nutrients, *i.e.*, nitrogen, phosphorus, potash, calcium, and magnesium.

Next to the check plots in low yields stand those receiving fertilizers without potash, nos. 4 and 6. In these cases both the yields and the percentage of nutrients in the alcoholic extracts of the leaves were higher than in the check plots, but considerably lower than in plots receiving complete fertilizers.

Plots receiving complete fertilizer mixtures (nos. 2, 3, 5, 7, and 8) showed the highest potato yields and also the highest content of total nutrients in the alcoholic extracts of the leaves.

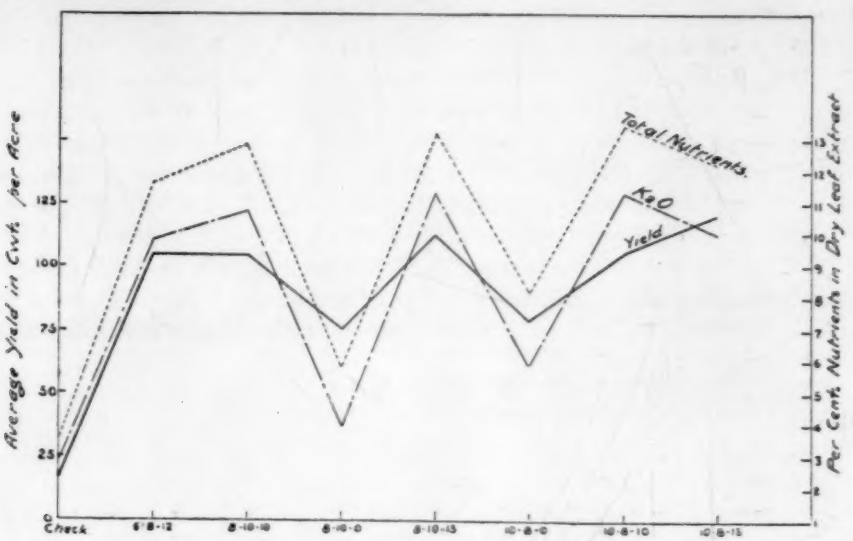

FIG. 1. Yield of potatoes in relation to potassium supply.

If the contents of the individual elements comprising the total nutrients are correlated with yields obtained, it will be seen that table IV presents very interesting information. The content of potash in the alcoholic leaf extracts was directly correlated with the amount of this element applied in the fertilizer and again with the yields obtained (fig. 1). The lowest content of potash resulted in the leaf extract of the check plots. The highest content of this nutrient was found in the leaf extracts of plots treated with complete fertilizers. Therefore, the application of potash in the fertilizer resulted in an increase of this nutrient in the alcoholic leaf extract and also in an increased yield. Treatments with no-potash fertilizers present an interesting case. Even though the amount of potash absorbed by the plant and actually found in the alcoholic leaf extract was considerably lower than in the case of plots receiving fertilizers with potash, it was higher than in the check plots. The crop yields followed the same sequence. They were lower than those resulting from the complete fertilizers but very much higher than the check plots. The crop received nitrogen and phosphorus, and these elements were absorbed by the plants and found in the leaf extracts in a higher proportion than in the check plots. It appears, therefore, that the mere absorption of more nitrogen and phosphorus stimulated the plants to absorb more potash. If this nutrient had been present in the soil in adequate amounts, the crop would have absorbed a larger amount to meet its requirements, or to balance the other elements.

It is also interesting to observe among the fertilized plots that the nitrogen absorption was not handicapped by withholding potash from the fertilizers. On the contrary, this element was evidently absorbed by the plant uninterruptedly and thus it is present in the alcoholic extract of the leaves, irrespective of the amount of potash present in the fertilizer.

Phosphorus, on the other hand, acts differently. Its absorption is apparently dependent on, or limited by, the amount of potash absorbed by the plant. This is shown in table IV by comparing columns representing the contents of both phosphorus and potash in the alcoholic extracts of potato leaves. For convenience these figures are plotted in figure 2. This graph shows plainly that as the content of potash in the alcoholic extract of the leaves increases, the amount of phosphorus in the extract also increases. When potash was withheld from the fertilizer, however, the contents of both potash and phosphorus in the alcoholic leaf extract remained low even though the phosphorus content of the fertilizer was maintained at a high level. These facts would indicate that phosphorus cannot be efficiently used by the potato crop if the potash supply of the soil is deficient.

The contents of both calcium and magnesium in the alcoholic extract of potato leaves bear an inverse relation to that of potash (fig. 3). The percentage of each of these elements tends to decrease as that of potash increases and *vice-versa*.

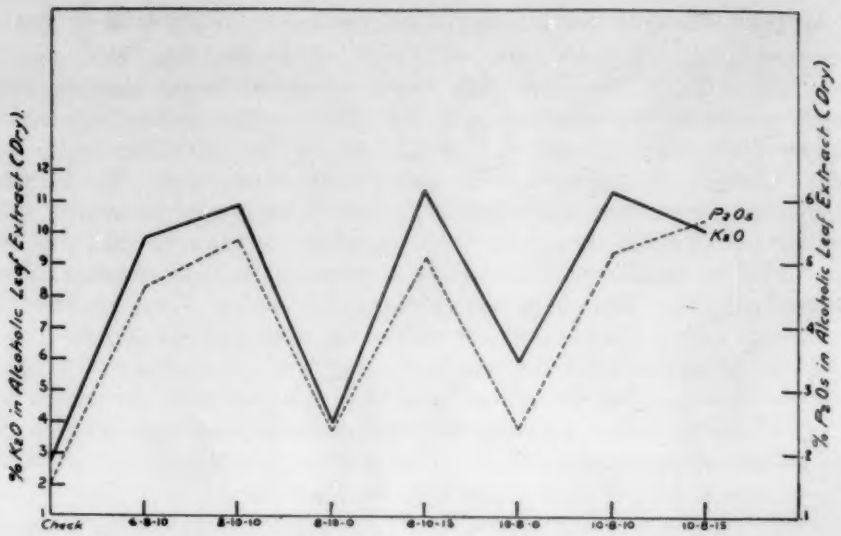

FIG. 2. Relationship between phosphorus and potassium in alcoholic extracts of potato leaves.

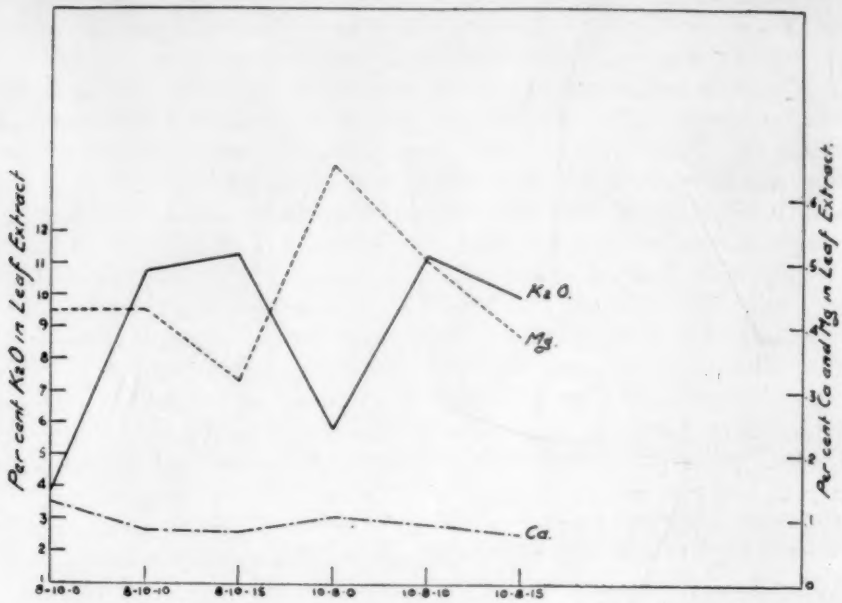

FIG. 3. Relationship between calcium, magnesium, and potassium in alcoholic extract of potato leaves.

QUANTITATIVE AND QUALITATIVE RELATIONSHIPS OF THE MINERALS IN THE ALCOHOLIC LEAF EXTRACTS TO CROP YIELDS

Table IV and figure 1 show that the total yield per acre is directly correlated with the total amount of minerals in the alcoholic extract of the leaves. The lowest yields invariably corresponded with the lowest percentages of total nutrients in the alcoholic extract of the leaves, and *vice-versa*, the highest yields obtained in treatments showed the highest percentage of total nutrients. These facts indicate that the percentage of total nutrients absorbed by the plant and concentrated in the leaves at the right time determines the productiveness of that plant at harvest time. Consequently, a quantitative relationship is involved between the amount of nutrients in the alcoholic leaf extract and crop yields.

The percentage of the different nutrients in the mineral portion of the leaf extract varies according to the different treatments. Table V shows the percentage composition of the mineral portion.

TABLE V

PERCENTAGE OF NUTRIENTS IN THE MINERAL PORTION OF THE LEAF EXTRACT

TREATMENTS	YIELD PER ACRE	K_2O	P_2O_5	N	CA	MG	TOTAL
	cwt.	%	%	%	%	%	%
1. Check	17.39	78.67	4.25	11.76	2.17	3.15	100.00
2. 6–8–10	105.28	83.96	3.95	8.37	0.82	2.90	100.00
3. 8–10–10	103.63	83.72	4.16	8.11	0.65	3.36	100.00
4. 8–10–0	75.67	68.57	4.02	17.86	2.18	7.37	100.00
5. 8–10–15	112.80	85.33	3.84	7.73	0.63	2.42	100.00
6. 10–8–0	79.43	71.72	2.91	16.04	1.30	8.03	100.00
7. 10–8–10	105.98	83.87	3.83	7.82	0.71	3.77	100.00
8. 10–8–15	120.79	81.62	4.65	9.89	0.65	3.19	100.00

From this table it is seen that the percentage of potash in the mineral portion of the leaf extract is lower in the check plots and in those receiving no-potash fertilizers. In the latter it is still lower than in the former. The percentage of potash was highest in plots receiving complete mixtures. In this case, there is little variation between treatments with 10 and 15 per cent. potash in the fertilizers.

The phosphorus content of the mineral portion is variable and is not consistent in relation to the different treatments. The lowest figure is found in plots treated with a no-potash mixture. It is highest in plots fertilized with the 10–8–15 formula. Among the remaining treatments it is more or less the same, but its content in the check plots is higher than in the latter.

The percentage of nitrogen in the mineral portion of the leaf extract is noteworthy. It appears to be highest in the check plots and in those

fertilized with the no-potash mixtures. It is considerably lower in plots receiving the complete fertilizer mixtures. The fact that the lowest yielding plots had a higher nitrogen content in the mineral portion of the leaf extract indicates that under a deficiency of potash this element is not completely assimilated and thus converted into plant tissues or fruits. Consequently, a state of equilibrium or balance of the mineral elements in the leaf is essential for an efficient metabolism.

The content of calcium in the mineral portion of the leaf extracts is much higher in the check plots and in no-potash plots than in those to which complete fertilizers were applied. In this respect calcium resembles nitrogen.

The amount of magnesium in the mineral portion of the leaf extracts also resembles that of calcium and nitrogen in that it is higher in plots receiving fertilizers without potash than in plots fertilized with the complete mixtures. In the check plots, however, the ratio of magnesium in the mineral portion of the leaf extracts did not increase to a relatively high figure as in the case of no-potash plots. It is rather within the limits of the figures found in the leaf extracts from plots receiving complete fertilizer mixtures.

Table V shows that the contents of both calcium and magnesium in the mineral portion of the alcoholic extracts of potato leaves vary inversely with the contents of potash in the leaves and in the fertilizer. When no potash was applied, the content of this nutrient in the mineral portion of the leaf extracts was relatively low and, in turn, the percentages of calcium and magnesium in the latter were relatively high. On the other hand, as the percentage of potash in the mineral portion of the leaf extract increased, the contents of calcium and magnesium decreased.

These facts give weight to the opinion of the writer, and other investigators, that an equilibrium between the minerals within the plant is essential for optimum growth and productivity. Moreover, this mineral equilibrium or plant food balance, shows both quantitative and qualitative relationships.

LAGATU and MAUME (**11, 12, 13, 14**), THOMAS (**22, 23**) and others (**19**) use the expression "intensity of nutrition" to represent the sum of the percentages of nitrogen (N), potash (K_2O) and phosphoric acid (P_2O_5) in dried foliage. This factor represents the quantity of nutrition at the moment of leaf sampling. Likewise they represent the quality of nutrition by the so-called "N–P–K unit." This unit is obtained by expressing in milligram equivalents the percentages of the three elements on the dried foliage, determining the proportion in which these elements are found in the total of their respective values and multiplying by 100 to avoid fractions. These two concepts, intensity of nutrition and N–P–K units, have been found to be intimately related with differential fertilizer treatments and their corresponding yields.

In order to confirm the importance and usefulness pertaining to the researches of LAGATU and MAUME, THOMAS, and their collaborators, their concepts of intensity of nutrition and N–P–K units are applied in this work to the mineral composition of the dry alcoholic extract of potato leaves although it is admitted that the above mentioned investigators base both magnitudes on the composition of the dried leaf.

In table VI are summarized the yields per acre, the intensity of nutrition and the N–P–K units as found in the dry alcohol leaf extract.

TABLE VI

YIELDS PER ACRE, INTENSITY OF NUTRITION, AND N–P–K UNITS IN DRY ALCOHOL LEAF EXTRACT

TREATMENTS	YIELD PER ACRE	INTENSITY OF NUTRITION	N–P–K UNITS		
		$N + P_2O_5 + K_2O$	N	P_2O_5	K_2O
	cwt.	%			
1. Check	17.39	3.454	31.15	6.67	62.18
2. 6–8–10	105.28	11.204	23.41	6.55	70.04
3. 8–10–10	103.63	12.336	22.81	6.92	70.27
4. 8–10–0	75.67	5.237	43.88	5.86	50.26
5. 8–10–15	112.80	12.826	21.87	6.42	71.71
6. 10–8–0	79.43	7.383	40.96	4.40	54.64
7. 10–8–10	105.98	12.835	22.26	6.47	71.27
8. 10–8–15	120.79	11.746	27.09	7.55	65.36

This table shows that there is a very high correlation between the yields of the different plots and the corresponding values expressing the intensity of nutrition and the N–P–K units as determined from the composition of the dry alcohol leaf extract. Taking the check treatment as a reference point, the above figures show that any fertilizer treatment resulting in an increased yield raised the intensity of nutrition and modified the N–P–K unit. Fertilizer treatments resulting in more or less similar yields show similar values for their intensity of nutrition and N–P–K units. Moreover, these figures indicate that both quantity and quality of nutrition are involved in the yield of the potato. Check treatments as compared with complete fertilizer treatments show a very low intensity of nutrition and an N–P–K unit having a higher proportion of nitrogen and a lower ratio of potash. No-potash treatments compared with check treatments show a higher intensity of nutrition but a highly unbalanced N–P–K unit, the K_2O content of which is lower but with a higher proportion of nitrogen. On the other hand, the addition of potash to the fertilizer resulted in a higher intensity of nutrition and a better balanced N–P–K unit, the latter having a higher K_2O content and a lower nitrogen content.

The facts derived from this investigation point to the following generalizations: An optimum yield in the potato is characterized by an optimum intensity of nutrition and an optimum equilibrium in the N–P–K unit as determined from the mineral composition of the dry alcohol leaf extract. Yields are lower than the optimum when the intensity of nutrition is low or when the N–P–K unit is unbalanced or when both of these conditions obtain in respect to the corresponding magnitudes for the optimum yielding plots.

Discussion

Several investigators (**11, 12, 13, 14, 20, 23, 24**) have found a correlation between the mineral composition of plant leaves, crop yields, and fertilizer applications. The works of LAGATU and MAUME (**11, 12, 13, 14**), MAUME and DULAC (**19**), THOMAS (**22, 23**), and others are outstanding in this respect.

In 1934, BEAUCHAMP, LAZO, and BONAZZI (**3, 4**) correlated the mineral composition of similarly located leaves from sugar cane plots, receiving differential fertilizer treatments, with crop yields. Both the alcohol-soluble and alcohol-insoluble leaf portions were analyzed separately. It was found that there was a definite correlation between the mineral content of the alcohol-soluble leaf portion and cane growth with its subsequent yield and the fertilizer treatments effected. On the other hand, the composition of the leaf skeleton or the alcohol-insoluble leaf portion did not show any such correlations.

In another investigation conducted by BEAUCHAMP and LAZO (**2**) on sugar cane, the mineral composition of the alcoholic extract of the leaves was found to be directly correlated with cane growth and yield. Direct correlations were found between the total mineral content found and cane growth as measured by average stalk length, average number of stalks per stool, average stalk weight, total cane weight and the percentage of available sugar in the juice. Increments of potash in the fertilizer resulted in increasing amounts of this element in the alcohol-soluble leaf portion and in turn, in increasing cane growth in terms of the above mentioned indices. On the other hand, increments of phosphorus or nitrogen in the fertilizer did not result in increasing amounts of these nutrients in the alcoholic leaf extracts or in a better response of the cane in the field.

This same method of alcoholic extraction was followed in the present study with the potato crop. It is well to consider that the succulent green potato leaves liberate considerable water to the alcoholic medium; the extracting liquid thus consists of a water-alcohol mixture. Other experiments are in progress to determine the effect of different ratios of water and alcohol on the efficiency of the mineral extraction. Further studies are also being conducted to determine other methods of extraction since

the Soxhlet process is time-consuming, especially when a large leaf sample is used.

The facts reported in this investigation show that the composition of the alcoholic extract of potato leaves is intimately correlated with crop yields and with the different fertilizer treatments applied. Analysis of the data obtained also confirms the results secured by LAGATU and MAUME (**11, 12, 13, 14**), MAUME and DULAC (**19**), THOMAS (**22, 23**) and others, working with entire leaves. These investigators found a definite correlation between the mineral composition of crop leaves, yields, and fertilizer treatments. Furthermore, the study here reported brings to light the antagonism, or reciprocal relationships, existing between various nutrients.

The yield of potatoes per acre varied directly with the total percentage of nitrogen plus phosphorus, potash, calcium and magnesium in the alcoholic extracts of the leaves. A low mineral content in the alcoholic extract corresponded with a low yield of potatoes per acre; a high mineral content corresponded with a high yield. This fact is important from a practical standpoint since it reflects the fertility level of the soil and thus the productive capacity of the crop grown in that soil. A low fertility level resulted in a low mineral content in the alcoholic leaf extract and *vice-versa*. Any significant yield increase resulting from a fertilizer application was correlated with a significant increase in the mineral content of the alcoholic leaf extract. Conversely, the mineral content of the latter did not change significantly when the fertilizer applied failed to produce a significant yield increase. Increments of potash in the fertilizer resulted in increasing percentages of this nutrient in the alcoholic extract and, in turn, in the yield of potatoes per acre. Neither nitrogen nor phosphorus increments in the fertilizer increased the amounts of these nutrients in the alcoholic leaf extracts, but they increased the content of potash in the latter as compared to the potash content of the leaf extracts of the check plots. This resulted in a higher nutrient content and hence in better yields than the check plots.

The percentage of phosphorus, however, in the alcohol-soluble leaf portion increased concomitantly with increases of potash in same, irrespective of the phosphorus content of the fertilizer. When no potash was applied, the content of both potash and phosphorus in the alcoholic extracts dropped to a very low level, despite the fact that the phosphorus content of the fertilizer remained unchanged. The absorption of nitrogen by the alcohol-soluble leaf portion was not affected by withholding potash from the fertilizer. These facts confirm the results obtained by BEAUCHAMP, LAZO, and BONAZZI (**3, 4**), and by BEAUCHAMP and LAZO (**2**), in the sugar cane investigations.

In the present study it is seen that increments of potash decreased the contents of both calcium and magnesium in the alcohol leaf extracts. This

agrees with the findings of numerous investigators on the question of antagonism (**1, 5, 6, 7, 9, 10, 15, 16, 17, 18, 20, 21, 25**). In an important research, THOMAS and MACK (**24**), found that there was a definite relationship between the contents of K_2O, CaO, and MgO within the leaves of corn and potato plants. When the content of potash increased, calcium and magnesium decreased, and *vice-versa*. These investigators arrived at the conclusion that when either of these elements is not present in sufficient amounts in the soil, the plant responds by a relatively excess absorption of one of the others, resulting in a disequilibrium which is reflected in reduced yields.

In the mineral portion of the alcohol leaf extracts, it is seen that nitrogen, calcium, and magnesium increase as the content of K_2O decreases. When no potash was applied the percentage of these elements in the mineral portion of the alcoholic extracts increased to very high levels but the resulting yields were low. On the other hand, when yields were high the content of potash in the mineral portion of the alcohol leaf extract was high while that of nitrogen, calcium, and magnesium was low. These facts indicate that an optimum percentage of potash in the alcohol-soluble leaf portion is essential for an efficient utilization of those three nutrients in plant metabolism.

The present investigation affords eloquent evidence as to the quantitative and qualitative relationships between soil fertility and crop yields as has been reported by numerous investigators. When the supply of plant nutrients in the soil was low the mineral content of the alcohol-soluble leaf portion was low and low yields resulted even if the ratio between the various nutrients departed little from normal. On the other hand, yields were also low when the plant food supply of the soil was unbalanced. In that case the ratio between the different nutrients in the mineral portion of the alcohol soluble leaf portion was abnormal.

The magnitudes representing the intensity of nutrition and the N–P–K units used by LAGATU, MAUME, and THOMAS quoted above as derived from the composition of the dried foliage were applied in the present instance to the composition of the dry alcoholic leaf extract and the values so obtained confirm the results of those investigators to the effect that both the quantity and quality of nutrition are involved in an optimum yield. Consequently, the analysis of the values so obtained afford an adequate means of correlating fertilizer treatments or soil fertility with crop yields.

The results of this investigation make it evident that the study of the mineral composition of the leaves, or different leaf portions, of cultivated crops may become a very useful tool in the hands of the agronomist or the plant physiologist to correlate soil fertility with crop yields thus formulating a sound basis for judicious fertilizer recommendations.

Summary

1. Increments of potash in the fertilizer resulted in marked increases in yield.

2. Increments of nitrogen and phosphorus did not produce any increase in yield.

3. The field yields of potatoes showed a very good correlation with the total percentage of plant food nutrients found in the alcoholic extracts of the leaves. Leaf samples were taken 52 days after planting, and harvesting was effected 16 days after sampling. A low total percentage of nutrients in the dry matter of the alcohol leaf extract always resulted in low yields, and conversely, a high percentage of total nutrients in same resulted in high yields.

4. The increase of potash in the fertilizer resulted in an increase in the percentage of this element in the alcohol leaf extract and, in turn, a higher yield. The lowest percentage of potash in the leaf extract occurred in the check plots, followed by no-potash plots. Yields showed the same sequence.

5. No-potash plots showed a higher content of potash in the leaf extract than did the check plots; indicating that the addition of nitrogen and phosphorus alone stimulated the plant to absorb more potash from the soil.

6. The percentage of phosphorus in the alcoholic leaf extract was not correlated with the amount of this nutrient applied in the fertilizer. On the other hand, it showed a marked parallelism with the amount of potash present in the alcohol-soluble portion of the leaves. The percentage of phosphorus in the alcoholic leaf extract was always low when the content of potash in the leaf was low, irrespective of the content of phosphorus in the fertilizer.

7. The percentage of nitrogen in the alcohol leaf extract did not present any correlation with the content of potash in the fertilizer.

8. The percentages of both calcium and magnesium in the leaf extracts showed an inverse relation to that of potash. They displayed a tendency to increase as the latter decreased and *vice-versa*.

9. The percentage of total nutrients in the mineral portion of the leaf extract (sum of $K_2O + P_2O_5 + N + Ca + Mg$) varied directly with the fertilizer treatment. In check and no-potash plots this value was lower than in plots receiving complete fertilizers.

10. The yield of potatoes in this investigation appeared to be correlated with the mineral composition of the dry alcoholic extract of the leaves. Both quantitative and qualitative relationships were shown, since the highest yielding plots showed a high mineral content and practically a definite ratio between the three major fertilizing nutrients; the lower yielding plots showed a lower mineral content and the ratio in which the

fertilizing elements were present departed from that found in the highest yielding plots.

Analyses for nitrogen, phosphorus, potash, calcium, and magnesium were run in duplicate by Mr. J. Mitchell of Purdue University under the direction of Dr. H. R. Kraybill who volunteered to cooperate in this investigation.

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SOME ASPECTS OF MINERAL NUTRITION IN RELATION TO BISON FLAX

E. A. Helgeson, T. H. Hopper, and David Taylor

(with three figures)

Introduction

That the yield and quality of oil produced by flax seed varies from year to year is a matter of common knowledge. In the absence of exact experimental data it is frequently stated that climatic factors are responsible for this variation. Since little is known about the actual process of oil synthesis by the flax plant and still less about the effect of specific environmental factors on this synthesis, it was felt that some careful studies under controlled conditions should be initiated. In these first studies the authors undertook to grow seed flax in selected nutrient solutions in an attempt to determine if there was any relation between mineral nutrition and the quality and quantity of the oil produced from flaxseed.

Fertilizers and their relation to yield of flax grown in the field have received some attention by various workers throughout America; little exists in the literature, however, relative to the growth of flax in nutrient solutions. Turner (**15**) studied the effect of variations in the nitrogen supply on the ratio of top to root growth of Winona flax. Using solutions high and low in nitrate nitrogen, he found that there was little difference in the nitrogen content of flax grown in these nutrients. He attributed the results obtained to relatively limited use of nitrogen by flax, and concluded that in many cases nitrogen fertilizers when applied to flax are not particularly beneficial.

After studying the effect of the light factor on cell production of fiber flax grown in sand culture, Robinson (**8**) reported that the tallest growth resulted when fiber flax was grown in ten hours of light per day; flax which was exposed to light eighteen hours each day matured earliest but produced the least vegetative growth. Thus the growth period of flax might be shortened by lengthening the photoperiod. His report showed that for fiber flax nitrogen was particularly important in early growth, and that potassium was more important as the plants matured. Adams (**1**) worked out the relation between duration of light and growth, with reference to flax grown in soil, and his findings in most instances bear out those of Robinson.

In Germany, Selle (**11**) made tests on soil acidity in relation to growth of fiber flax. He noted that the yields, based on fiber, were greatest on lime-rich soils and poorest on acid soils. Potassium, according to Powers (**7**), was of the greatest importance in fertilizers for fiber flax; its presence

in the soil increased strength and length of fiber and vigor of growth. Nitrogen was second in importance. Potash was required relatively early in order to obtain maximum benefits.

Fiber flax was grown in liquid nutrient media by SHKOL'NIK (**12**). He worked especially with boron, manganese, aluminum, and copper. Boron was found to be of paramount importance, its absence causing a poor development of the root system and eventual death. The optimum concentration of boron was 0.5 mg. per liter; doses of about 5 mg. per liter were toxic. Manganese and other elements mentioned appeared less important. A few years ago SOMMER (**13**) showed that the addition of small quantities of copper to nutrients supporting flax growth resulted in increased vigor as compared to growth of plants in solutions lacking that element.

SCHMALFUSS (**9**) grew fiber flax in pots of loamy soil, the water content of which was controlled, and which received various fertilizer treatments. He reported the following findings: lignification of fiber cells was increased by lowering the water content of the soil, and also by increasing the amount of sulphate ion added; lignification was decreased by increasing the amount of chloride ion; the fiber content of the stem was increased by nitrogen, and more so by K_2O; the fiber content was increased by the sulphate ion, and decreased by the chloride ion. He further reported that the yield and quality of oil produced were affected by fertilization. In this respect the iodine number of oil from nitrogen-starved plants was highest. The iodine number was affected more by anions than by cations; the chloride ion increased and the sulphate ion decreased the iodine number of the oil produced. His results showed that the nitrogen content of the seed varied inversely with the oil content. The observed reactions on amount and quality of fiber and oil he explained as results of effects of the above mentioned factors on the colloids, hydration of the plant cells, and the water economy of the plants.

No attempt will be made here to present an extended review of the literature on the subject of physiological nutrient solutions or on the technique of nutrient culture work. TOTTINGHAM (**14**) gave a thorough review of the subject in his paper of 1914, and since then a number of workers have dealt with various other phases. The triangle system as used in this work has been adequately discussed by SCHREINER and SKINNER (**10**) and MILLER (**6**).

Preliminary experiments were carried out in the winter of 1935–36 in order to find the approximate concentration of nutrients that would support the growth of flax. In these tests a pure line of Bison flax, developed by H. L. BOLLEY and O. HEGGENESS of the North Dakota Agricultural Experiment Station was grown to the flowering stage in SHIVE's type I, three-salt, liquid nutrient solutions. The best growth was obtained in solutions

having a composition of 25 to 37.5 per cent. KH_2PO_4, 50 to 62.5 per cent. $Ca(NO_3)_2$, and 25 to 37.5 per cent. $MgSO_4$, based on the total salt concentration of the nutrient solution.

Methods and materials

During the winter of 1936–37 Bison flax was grown to maturity in ten of SHIVE's type I, three-salt liquid nutrient solutions. These solutions were chosen so as to be well distributed over the triangle; they included solutions R1S5, R2S1, R2S3, R2S4, R2S5, R3S2, R3S3, R4S2, R4S3, and R5S2. Partial volume molecular concentrations are given in table II.

The necessary elements for plant growth are supplied in type I, three-salt solutions by KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$. In making up the nutrients these salts were added from M/2 stock solutions to distilled water in the proportions prescribed by the committee of Biology and Agriculture of the National Research Council in its 1919 report. The osmotic values of all solutions were determined cryoscopically and were found to be somewhat under one atmosphere.

Equal amounts of iron, manganese, and boron were added to each nutrient solution. The iron was added as soluble ferric phosphate from a 0.0134 M stock solution, 2 ml. of this solution being added to each liter of nutrient. One-half part per million of $MnSO_4$ and H_3BO_3 was added to each liter of nutrient solution prepared. All chemicals used in preparing the nutrient solutions were of C. P. analyzed grade secured from the J. T. Baker Chemical Company. Fresh solutions were prepared and added to the cultures daily.

A constant renewal system (fig. 1) was set up and regulated to feed, at the rate of 1.5 liters of solution per day, into each series of cultures. In this system the fresh nutrient was added to the upper reservoir, and from there it passed through a constant-level siphon, into the lower reservoir. The nutrient then flowed through the delivery siphon, dropped into the intake tube, and flowed down the tube into culture A. When the level of solution in culture A exceeded that in the delivery arm of the siphon, the solution was siphoned from culture A into culture B; the same process occurred in cultures B and C until the nutrient solution passed from that set of cultures into the drain. Each drop of nutrient, upon falling into the delivery tubes, would trap an air bubble, which was driven down through the intake tube, aerating the nutrient solution as it rose to the surface in each culture jar. A shield of insulation board was constructed to cover the reservoirs in order to prevent heating of the solutions in the reservoirs by the sun's rays. The term "bank" has been used to designate the three culture jars of a series. The two one-gallon reservoirs of each bank were closed with rubber stoppers and painted to exclude light, as was all glassware used in the set-up.

The paraffined stoppers, used to support the plants, were anchored in tin, two-quart Economy jar covers which were heavily coated with paraffin. Ten such banks, each composed of two reservoirs and three cultures, were used in the experiment. These banks remained in a fixed position during the entire study.

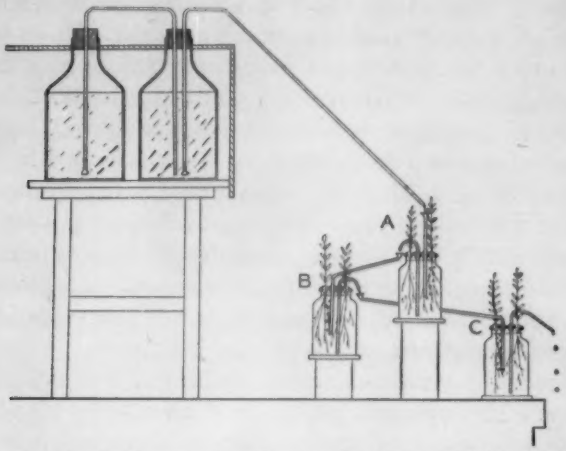

FIG. 1. Constant renewal system used in flax nutrition studies.

Seed of the Bison variety from the same lot used in the 1935–1936 studies was used in this experiment. Quantities of seedlings were obtained using methods similar to those described by HOAGLAND and BROYER (4). When the tops of the seedlings were 3 cm. long, uniform seedlings were selected, their stems were wrapped in cotton at the base and enclosed in a cork. Five seedlings were placed in each culture container. Cultures were set up on November 27, 1936. The plants were supported by a network of threads. The temperature of the greenhouse was thermostatically controlled at 18 to 20° C.; the relative humidity generally ranged from 40 to 50 per cent.

Throughout the experiment the plants were exposed to 24 hours of light each day. At night light was supplied by four 200-watt Mazda bulbs equipped with porcelain reflectors. As measured by the Weston Photronic cell a light intensity of from 130 to 140 foot-candles was obtained all along the table at the tops of cultures of each bank.

Hydrogen ion determinations were made on the freshly prepared nutrient solutions and the solutions leaving the different banks on three different days during the period of the experiment using both the Youden quinhydrone cell and colorimeteric procedures. In all cases these tests were conducted according to the directions given by CLARK (3).

All cultures were allowed to grow to maturity or until the main stems of the plants had completely dried. Upon removal of the plants from the

cultures, data on oven-dry weight of roots and tops were taken on the five plants from each culture. Dry weights were determined on material dried to constant weight at 70° C. After the seeds had been removed, fruits were hand picked and threshed, the seed from the main stems being kept separate from that of the lateral branches. A record was made of main stem, lateral, and total seed in each culture; and the weight of one hundred seed samples of the main stem seed was also recorded.

The flaxseed samples were stored togther with a bulk sample and all assumed to acquire the same moisture content as the bulk sample. Moisture determinations were run on the bulk sample since the experimental seed lots were too small to permit of such determinations. Each entire experimental lot was then weighed and thoroughly ground with round sand in a porcelain mortar. The ground seed and sand was carefully washed with Skelly Solve F into a special glass extraction thimble and extracted for 24 hours in a percolation extractor with the same solvent. The quantity of oil was determined by direct weighing of the oil in the tared extraction flask after removal of the solvent and drying in a vacuum oven for one half hour at 110° C. The iodine number of the extracted oil was determined refractometrically according to the method of HOPPER and NESBITT (5).

Total nitrogen was determined on the extracted residue by the official A.O.A.C. method.

Results and discussion

The hydrogen ion concentrations of the nutrient solutions were determined on freshly prepared solutions on January 17, February 28, and March 26. All of the fresh nutrients had pH values between 5.7 and 6.0. Likewise, the solutions coming out of the different culture jars were tested

TABLE I

CHANGES IN HYDROGEN ION CONCENTRATION OF NUTRIENT SOLUTIONS BROUGHT ABOUT BY THE GROWTH OF FLAX

SOLUTION AND DATE OF TEST	pH VALUE*									
	NUTRIENT NUMBER									
	R1S5	R2S1	R2S3	R2S4	R2S5	R3S2	R3S3	R4S2	R4S3	R5S2
Fresh solution	6.05	5.85	5.85	5.75	5.85	5.75	5.75	5.75	5.75	5.65
Used solution 1/17/37	5.9	5.7	5.75	5.75	5.75	5.65	5.6	5.65	5.6	5.65
Used solution 2/28/37	5.35	5.35	5.45	5.45	5.65	5.65	5.45	5.25	5.55	5.55

* Average of three cultures in bank.

on January 17, and February 28, fifty-one and seventy-five days, respectively, after the seedlings had been placed in the culture jars (table I). At the time the used solution was first tested, the plants were just entering the period in which they made most marked and rapid growth. The pH of solutions coming from the different cultures of a bank was uniform, and showed little change from that of the freshly prepared nutrients. The second test was made after the plants had attained a considerable size and were still growing rapidly. The results in the second test showed that the pH of the nutrients varied slightly as they left the different cultures of a bank; there was a general tendency, however, for the solutions to become slightly more acid as they flowed through the banks.

These changes seem to be in no way correlated with the original composition of the nutrient solutions. All solutions were uniform in that they tended to become more acid. CHIZHERSKAIA (**2**) carried on water culture experiments to determine the relation of sprout growth in flax to the pH of the nutrient medium. From these experiments she found that the maximum sprout growth occurred at pH 5, and pH 9. It must be remembered that her conclusions on acidity relations were based upon the early growth of flax, while in the experiments reported above large plants were involved.

Considering the nutrients on the basis of the length and dry weight of tops produced, it is evident that a slightly greater growth resulted in cultures containing relatively high concentrations of KH_2PO_4 (table II). In general there was no region of the triangle used where nutrients were definitely superior in respect to height and dry matter produced. There is no indication of a clear cut correlation between the composition of the nutrient solution and the final length of top growth; the cultures which were slowest to mature, however, R1S5 and R5S2, made the tallest top growth. Plants in all cultures were considerably taller than field grown plants of the same variety. According to the records available in this Experiment Station, this same strain, over a period of years, has produced plants ranging from 40 to 61 centimeters in height (data obtained from H. L. BOLLEY and O. HEGGENESS). The data on dry-matter produced can be considered only as relative owing to the fact that dry-weights could not be taken until the plants had matured, with a consequent loss of some leaf material. Yields in regard to dry-weight were rather definitely related to the concentration of KH_2PO_4, the largest amount of dry-matter being obtained in solutions with the highest concentration of that salt. Concentration of other salts in these high KH_2PO_4 cultures varied considerably. The data appear to indicate that this variety of flax is not a heavy user of nitrogen.

The date of maturity of plants growing in the different solutions showed a certain degree of relationship to the amount of KH_2PO_4 present. Those solutions relatively high in KH_2PO_4 produced the earliest maturing plants.

TABLE II

COMPOSITION OF SELECTED SHIVE'S TYPE I NUTRIENT SOLUTIONS, VEGETATIVE GROWTH, SEED PRODUCTION, AND CHEMICAL ANALYSIS OF SEEDS PRODUCED IN THESE SOLUTIONS

PARTIAL VOLUME MOLECULAR CONCENTRATIONS OF SOLUTIONS USED					DATA ON VEGETATIVE GROWTH AND SEED PRODUCTION*					DATA FROM CHEMICAL ANALYSIS			
BANK NO.	SOL'N NO.	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	GROWTH PERIOD	HEIGHT OF TOP	DRY MATTER PER PLANT**	AVERAGE TOTAL SEED PER PLANT†	AVERAGE WEIGHT PER 100 MAIN-STEM SEEDS	PERCENTAGE OIL CONTENT	IODINE NUMBER	PERCENTAGE CRUDE PROTEIN ($N \times 6.25$)	
												MEAL BASIS‡	OIL AND WATER FREE BASIS
					days	*cm.*	*gm.*	*gm.*	*gm.*	%		%	%
1	R1S5	0.0022	0.0108	0.0043	185	119.4	2.566	0.428	0.641	36.6	164	27.6	47.74
2	R2S1	0.0053	0.0027	0.0132	169	106.7	2.267	0.519	0.633	36.7	174	25.7	44.63
3	R2S3	0.0047	0.0071	0.0071	159	109.2	2.378	0.611	0.663	37.2	174	25.3	44.27
4	R2S4	0.0045	0.0090	0.0045	161	95.3	1.724	0.453	0.655	37.9	175	24.8	43.88
5	R2S5§	0.0041	0.0104	0.0021	149	97.8	1.487	0.342	0.636	37.2	175	27.9	48.84
6	R3S2	0.0072	0.0048	0.0072	149	111.8	2.249	0.695	0.672	37.7	181	24.8	43.86
7	R3S3	0.0068	0.0068	0.0045	177	99.0	2.899	0.719	0.661	36.1	172	26.7	45.85
8	R4S2	0.0094	0.0047	0.0047	177	106.7	3.037	0.697	0.686	36.6	175	26.9	46.67
9	R4S3	0.0090	0.0068	0.0022	174	109.2	2.927	0.655	0.660	36.3	177	26.9	46.49
10	R5S2	0.0118	0.0047	0.0023	183	120.7	3.248	0.672	0.662	36.1	174	27.1	46.58

* Average value per plant from 15 plants in all three cultures of each bank.
** Exclusive of chaff.
† Total lateral and main-stem seed.
‡ Moisture assumed at 5.68% on basis of other samples stored under same condition and analyzed.
§ Figured on basis of culture 1 only.

Plants in high-nitrogen cultures tended to be more vegetative. Cultures high in KH_2PO_4 and relatively high in nitrogen were intermediate as regards maturity. Magnesium sulphate seems to have played a less positive rôle as far as length of growth period is concerned. The character of growth is shown in figures 2 and 3.

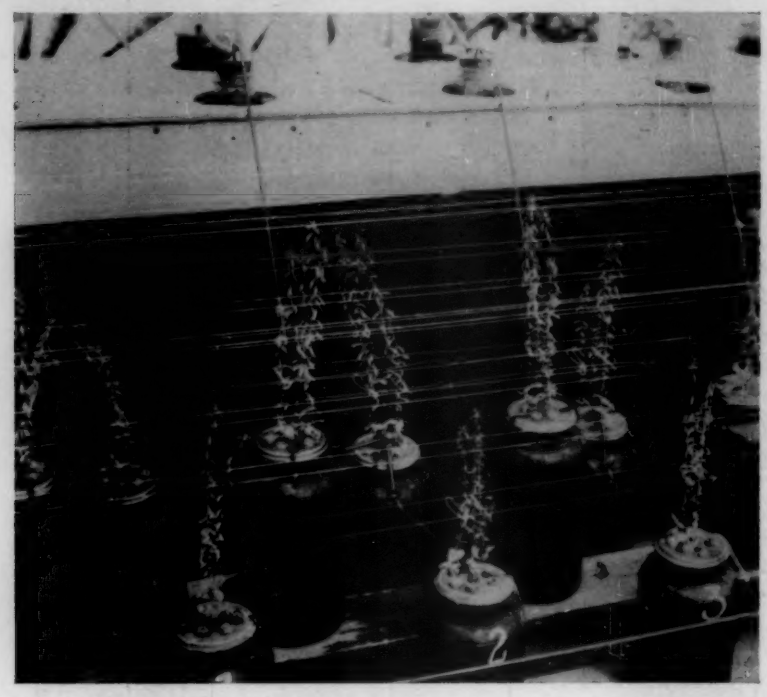

FIG. 2. The appearance of the top growth in the cultures of banks 1, 2, and 3 on January 1, 1937.

From the standpoint of total weight of seed produced, solutions medium to high in KH_2PO_4 again showed the best yields (table II). The presence of medium to high concentrations of the nitrogen-carrying salt seemed to depress the total yield of seed unless the amount of acid phosphate present was also fairly large. Data obtained on the yield from plants growing in the field showed those plants had produced, over a period of years, about 1 gram of seed per plant. In this experiment the yields in the different cultures ranged from 0.342 to 0.719 gm. per plant which was considerably less than the yields obtained from field grown flax of this variety.

The weight of 100 average seeds produced on the main stems of the plants in the different nutrient media ranged between 0.633 and 0.686 gm. Seeds from a large number of samples of Bison flax that were grown in the

FIG. 3. The appearance of the top growth in the cultures of banks 1 and 2 on March 25, 1937.

field in different localities varied from 0.318 to 0.677 gm. per 100 seeds and averaged about 0.533 gm. per 100 seeds. Our seed with an average of 0.657 gm. per 100 seeds was generally heavier than seed from this variety grown on plants in the field. The general tendency was toward heavier seed in cultures relatively high in KH_2PO_4; whereas plants grown on the nitrogen-high nutrient tended to produce lighter seed. All seed produced was, however, surprisingly uniform in weight (table II).

Analysis of the seed produced during this study showed that the yield of oil was about equal to that of the average field-grown seed, and that the iodine number of the oil was comparable to the highest of oil produced in field-grown flax at this station (table II). The amount of oil produced with the different nutrient solutions was rather constant, varying from 36.1 to 37.9 per cent., when calculated on a 5.68 per cent. moisture basis. Commercial samples of Bison varied from 32.0 to 40.0 per cent. with an average of 37.0 per cent. when calculated on the same basis. Tests for iodine number, made on the seed harvested from plants grown in this experiment, gave a range of from 164 to 181. Oil having the highest iodine number was produced in cultures well supplied with potassium acid phosphate. Data from the records on field tests conducted at Fargo show oils from flax-seed produced on Bison flax grown in the field had iodine numbers of 161 to 167. These findings indicate that the degree of saturation of linseed oil is not a fixed character but varies with the nutrient supply available.

The protein content of the seeds from the main stems of plants ran uniformly high in all cases, ranging from 38.36 to 49.63 per cent, on an oil and water free basis. Other experimental samples from field grown material have been found to vary from 28.4 to 53.4 per cent. crude protein on the same basis. Cultures high in $Ca(NO_3)_2$ and fairly high in KH_2PO_4 produced the highest yields of protein although the total difference was not great. Considering the yield of oil in relation to the protein content in the seeds one finds a fairly definite inverse relationship. It will be seen from table II that in some cases the protein content of the seeds decreased upon increase in oil content. This correlation bears out the findings of SCHMALFUSS (9) regarding the relation between nitrogen fraction and oil content.

Summary

In this study a pure line of Bison flax was grown to maturity in selected SHIVE's type I, three-salt nutrient solutions using a constant renewal technique. Temperature, humidity, and light conditions under which the plants grew were recorded. The data obtained on plants grown under the conditions of this experiment indicate that:

1. Final growth in height of top is relatively constant over a wide range of nutrient salt proportions.

2. The presence of fairly large quantities of KH_2PO_4 in the nutrient solutions resulted in relatively early maturity, the date of maturity being modified by the nitrogen content of the nutrient solution. The time of maturity seemed to be in inverse proportion to the amount of nitrogen present.

3. Relatively greater amounts of oven-dry material were obtained with nutrient solutions high in KH_2PO_4. The other constituent salts of the

various nutrient solutions showed no uniform relation to the oven-dry weight of plant material produced.

4. Generally the greatest seed production occurred in solutions containing the higher proportions of KH_2PO_4; other nutrient constituents show no definite relation to this criterion. The heaviest seed was obtained from plants which grew on nutrients relatively high in the potassium acid phosphate salt.

5. There is no marked variation in quantity of oil produced when the composition of the nutrient solution is varied over a rather wide range.

6. Oils from seeds produced in cultures relatively high in KH_2PO_4 have higher iodine numbers than those from cultures low in this salt.

7. The production of plants grown on nutrient solutions in constantly renewed solution cultures exceeded production of field-grown plants in the following respects: height of top, weight of seed, average protein content of seed, and iodine number of oil. The oil yields in the experimental plants about equal average oil yields of field grown plants.

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AUXITHALS SYNTHESIZED BY SOME FILAMENTOUS FUNGI[1]

LEON H. LEONIAN AND VIRGIL GREENE LILLY

Introduction

Since 1869 when RAULIN reported that *Aspergillus niger* excreted some growth-promoting substances into its medium, a number of investigators have studied the phenomenon of auxithal synthesis by fungi. For a comprehensive review of this the reader is referred to FRIES (2) and SCHLENKER (7). The writers (5) have referred briefly to this subject; the present paper reports a more exhaustive study.

The following six organisms were used as sources of auxithals: *Rhizopus suinus, Fusarium niveum, Phytophthora erythroseptica, Phycomyces blakesleeanus, Pythiomorpha gonapodioides,* and *Mucor ramannianus.* The first two fungi thrive in synthetic media without the addition of any growth substance; *Phytophthora erythroseptica* cannot grow without thiamin; *Phycomyces blakesleeanus* grows either in the presence of thiamin or of a mixture of pyrimidine and thiazole (thiamin moieties), *Pythiomorpha gonapodioides* grows in the presence of pyrimidine alone as well as in the presence of thiamin; *Mucor ramannianus* requires only thiazole for growth, but it does very well in the presence of thiamin.

Materials and methods

The nutrient solution employed by the writers consisted of the following: 0.5 gram each of ammonium nitrate, magnesium sulphate and potassium dihydrogen phosphate, ROBBINS and KAVANAGH'S (6) modification of HOAGLAND'S A–Z mixture of rare elements, 1 gram of a mixture of amino acids (d-arginine, d-glutamic acid, l-aspartic acid two parts each; dl-a-alanine, and glycine one part each), 5 grams Bacto dextrose, and 1000 ml. distilled water. The pH of the medium was adjusted to 5.5 with sodium hydroxide. Thiamin or its moieties were used at the rate of 1:20 million. The medium in which *Rhizopus suinus* and *Fusarium niveum* were grown contained neither thiamin nor its moieties; *Phytophthora erythroseptica* was grown in the presence of thiamin, *Phycomyces blakesleeanus* in the two moieties, *Pythiomorpha gonapodioides* in pyrimidine only, and *Mucor ramannianus* in thiazole only.

Fernbach flasks of 2800-ml. capacity were used for the cultures; each flask contained 500 ml. of the nutrient solution. The fungi were allowed to grow at room temperature and in diffused light until a mycelial mat formed on the surface of the medium. *Phytophthora erythroseptica* required 25

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days to reach this stage of development, *Mucor ramannianus* and *Fusarium niveum* two weeks, and the others 10 days.

The mycelium was filtered off, well washed with water, air dried at 30° C., extracted with water by autoclaving ten minutes at 15 pounds pressure, filtered, taken to dryness *in vacuo*, and added to the basic medium at the rate of 0.1 per cent. The filtered medium was treated with activated charcoal (Norit), eluted with pyridine-water (2:1), the solvent removed under diminished pressure, and the residue added to the basic medium at the rate of 0.1 per cent. Two per cent. agar was added to the medium which was then adjusted to pH 5.5, tubed, sterilized, and inoculated. After growing for two weeks at 25° C. the readings were taken.

Forty fungi were used to determine the auxithals present in the mycelium and the medium of the six fungi. Twenty-four of these organisms can grow only when thiamin or its moieties are added to the basic medium, while the remaining 16 do not respond significantly to thiamin or its moieties but must have biotin. The writers do not wish to convey the impression that thiamin or its moieties alone, or biotin alone, induce *optimum* growth in their respective groups of fungi because other chemicals and physical factors also may be accessory. For instance, *Acanthorhynchus vaccinii* grows fairly well in the presence of thiamin alone but produces a much richer growth when biotin is added; *Ashbya gossypii* makes only a slight growth when thiamin, biotin, and inositol are used separately, but when these three are added to the same medium a rich growth follows. Furthermore, organic acids, mixtures of different sugars and of nitrogen compounds, reducing substances, surface area and depth of the medium, higher concentrations of foods, etc. greatly influence the amount of growth. Organic compounds contain other and as yet unknown substances that bring about increased growth. For instance, grains, fungus extracts, and animal excreta induce growth increases far beyond the limits of their thiamin and biotin content.

The term biotin is used here reservedly as we have only circumstantial evidence that the specific auxithal responsible for the growth of certain organisms is biotin. KÖGL and TÖNNIS (3) thus far are the only investigators to have obtained biotin in pure form. KÖGL and FRIES (4) found that *Ashbya gossypii* and *Lophodermium pinastri* need biotin among other things before they can grow on synthetic media. The organisms listed by the writers as requiring biotin respond to the same auxithals as *Ashbya gossypii* and therefore are tentatively listed as organisms responding to biotin.

Discussion

Table I gives the response of biotin-requiring organisms. Roman numerals indicate the relative amount of growth; thus, 0 indicates no growth; I and II, trace; III, slight; IV and V, poor; VI and VII, fair; VIII, good;

X, excellent. A number of organisms can make a thin, submerged growth in the absence of auxithals, while upon the addition of such substances they grow profusely and the aerial mycelium fills the test tube.

The foregoing table (table I) shows that all six of the test fungi are capable of synthesizing biotin, a larger part of which is retained in the mycelium but considerable quantities diffuse into the medium. *Mucor ramannianus* stores up very small amounts of biotin and gives off into the medium still smaller quantities.

Organisms that are dependent upon extraneous sources of biotin require different amounts of it for optimum growth. For instance, *Sordaria fimicola* grows and reproduces very readily in the presence of the extract of *Fusarium niveum,* whereas *Allomyces javanicus* makes a slight growth; in fact it does not grow well in the presence of any of the extracts. It is possible, however, that the factor which limits the growth of this fungus may not be biotin but some sugar or some nitrogen compound. Our strain of *Saccharomyces cerevisiae* responds well to minute amounts of biotin while many others do not.

Because the hyphal and mycelial extracts used here are complex substances and contain, in addition to biotin, inositol, and thiamin, many other compounds some of which may act as auxithals, it will not be possible at this time to determine more closely the specific responses of different fungi to biotin alone.

Synthesis of thiamin by the six test fungi

The 24 organisms listed in table II require thiamin for growth. When transferred to the basic medium containing neither thiamin nor its moieties, they make either no growth, or form very sparse, submerged hyphae. Upon the addition of crystalline thiamin they all thrive and produce a rich aerial mycelium. Since the various fungal extracts replace thiamin, as can be seen in the following table, and no other known compound can bring about growth, it is assumed that the growth-promoting substance present in the mycelium of the six fungi tested consists of thiamin.

Table II shows that the extract from the medium is a poorer source of thiamin or its moieties than is the extract from the mycelium. Such organisms as *Lenzites saepiaria, Phytophthora erythroseptica, Pythiomorpha oryzae, Pythium ascophallon, Thielaviopsis basicola,* etc., that are unable to utilize thiazole and pyrimidine, alone or in mixture, but must have the thiamin molecule, failed to thrive in the medium extract of all six organisms. However, the organisms capable of utilizing thiazole and pyrimidine alone or in mixture made a good growth in the medium extract of all organisms except that of *Mucor ramannianus.* This indicates that the growing mycelium absorbs all the thiamin from the medium and retains it as in case

TABLE I

THE EFFECT OF THE MYCELIUM EXTRACT AND MEDIUM EXTRACT UPON THE GROWTH OF BIOTIN-REQUIRING ORGANISMS

Organisms	*Rhizopus suinus*		*Fusarium niveum*		*Pythiomorpha gonapodioides*		*Mucor ramannianus*		*Phycomyces blakesleeanus*		*Phytophthora erythroseptica*	
	Hyphal extract	Medium extract	Hyphal extract	Medium extract	Hyphal extract	Medium extract	Hyphal extract	Medium extract	Hyphal extract	Medium extract	Hyphal extract	Medium extract
Acanthorhynchus vaccinii	VIII	IV	VIII	VIII	VII	VII	V	III	VII	VII	X	V
Allomyces javanicus	V	0	IV	0	III	0	I	0	II	0	V	0
Ashbya gossypii	VI	0	VIII	I	VI	I	I	I	V	I	VII	I
Ceratostomella multiannulata	X	III	X	II	X	II	III	I	X	V	X	IV
Dipodascus uninucleatus	X	0	VIII	I	III	IV	III	III	VII	VI	VIII	IV
Lophodermium pinastri	VII	II	VI	II	VII	II	II	II	VIII	VI	X	II
Ophiobolus oryzinus	X	0	X	II	X	I	III	II	X	II	X	V
Ophiostoma catonianum	X	V	X	II	VI	II	II	II	X	II	V	0
Pseudopeziza ribis	VII	II	VII	II	V	IV	II	II	VII	II	V	II
Rosellinia arcuata	X	III	X	II	X	III	IV	II	X	II	X	VIII
Saccharomyces cerevisiae	VIII	VII	X	VIII	VII	VII	VI	VI	X	VII	X	VIII
Saccobolus depauperatus	VII	III	VII	III	VII	VI	IV	II	VII	IV	V	III
Sordaria fimicola	X	VII	X	X	VI	IV	X	IV	X	III	X	VIII
Sporormia intermedii	X	I	V	II	V	I	II	II	VIII	I	X	IV
Thraustoteca clavata	VII	I	VI	I	VI	I	V	I	VII	I	VII	I
Trachysphaera fructigena	VIII	VI	VII	VII	VII	VI	V	IV	X	VIII	VI	II

TABLE II

THE EFFECT OF THE MYCELIUM EXTRACTS AND THE MEDIUM EXTRACTS UPON THE GROWTH OF THIAMIN-REQUIRING ORGANISMS

ORGANISMS	*Rhizopus suinus*		*Fusarium niveum*		*Pythiomorpha gonapodioides*		*Mucor ramannianus*		*Phycomyces blakesleeanus*		*Phytophthora erythroseptica*	
	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT
Blakesleea trispora	X	VIII	X	VIII	X	X	VIII	VI	X	X	X	X
Chaetocladium brefeldii	VI	II	VII	III	VII	IV	II	I	X	VI	X	X
Collybia tuberosa	VII	V	X	II	X	VII	II	I	X	X	VIII	VII
Coprinus lagopus	X	III	VII	II	VII	IV	II	I	VII	IV	VI	III
Coprinus turgiversans	VII	V	X	II	X	X	I	I	X	X	X	X
Cyathus striatus	VIII	V	VI	IV	VIII	VII	III	II	VII	VI	VII	IV
Deconica inquilina	VIII	II	VII	II	VIII	II	II	II	VIII	II	VII	II
Dermatia balsamea	V	II	IV	II	V	V	III	II	V	V	V	IV
Gloeocystidium rosco-cremeum	X	V	X	I	V	I	III	I	VIII	I	X	I
Lentinus tigrinus	X	VII	X	VIII	X	X	VIII	VII	X	X	X	X
Lenzites saepiaria	VIII	0	X	0	V	0	0	0	X	0	VI	I
Lenzites betulina	X	II	X	III	X	VIII	III	II	X	VIII	X	VIII
Mucor ramannianus	X	VIII	X	X	VIII	VIII	VIII	VII	X	X	X	X
Phycomyces blakesleeanus	X	I	X	II	X	IV	III	II	X	VIII	VIII	VI
Phytophthora erythroseptica	X	0	X	0	X	0	III	0	X	0	X	I
Phytophthora fagopyri	X	VI	X	IV	X	X	VII	V	X	X	X	X
Pilaira moreaui	X	II	X	II	IV	II	IV	II	X	VIII	X	IV
Pythiomorpha gonapodioides	X	V	X	IV	X	X	V	III	X	VIII	X	VI
Pythiomorpha oryzae	VI	0	VI	0	VI	I	I	0	VI	0	VIII	I
Pythium ascophallon	X	0	X	0	X	I	I	0	X	0	X	I
Pythium oligandrum	X	II	X	I	VII	II	I	I	X	IV	VIII	V
Pythium polymastum	X	0	X	0	VI	III	I	0	X	0	X	V
Thielaviopsis basicola	X	0	VI	I	VI	I	IV	I	VII	I	X	II
Typhula variabilis	X	VIII	X	X	X	X	VII	V	VI	VII	X	X

of *Phytophthora erythroseptica*, giving off into the medium the first disintegration products of thiamin—the thiazole and the pyrimidine compounds. In case of *Phycomyces blakesleeanus* the mycelium absorbs pyrimidine and thiazole from the medium, converts them into thiamin, some of which is stored, and the balance is used up. This utilization of thiamin by the fungus results in the production of pyrimidine and thiazole which diffuses into the medium. It is not probable that the thiazole and pyrimidine content of the extract of Phycomyces medium is the original material added to the solution because a demand on the part of the protoplasm of the mycelium for thiamin would eventually drain these substances from the medium just as *Phytophthora erythroseptica* drained all thiamin from its medium.

The following experiment was made in order to demonstrate this: thiamin was substituted for pyrimidine and thiazole in the nutrient solution which was inoculated with *Phycomyces blakesleeanus*. After a growth of 10 days at 25° C. the mycelium was filtered off and the medium treated and tested as before. It contained no thiamin as shown by the failure of *Phytophthora erythroseptica* to grow, but enough pyrimidine and thiazole to induce growth in *Phycomyces blakesleeanus*, *Pythiomorpha gonapodioides*, and *Mucor ramannianus*. Since *Phycomyces* is capable of synthesizing thiamin from the moieties, the thiazole and the pyrimidine given off into the medium would have to be absorbed sooner or later and utilized. This was found to be the case by the following experiment: the fungus was grown in flasks of 250-ml. capacity, containing 25 ml. of the medium plus 1/100 p.p.m. of the moieties. At regular intervals the mycelium was filtered off, the medium solidified with agar and tested. It was seen that eventually all the thiazole content of the medium was reabsorbed but some of the pyrimidine was left as shown by the failure of *Mucor ramannianus* to grow at all and by the ability of *Pythiomorpha gonapodioides* to grow. On the other hand, the mycelium will contain some thiamin so long as it lives. This was demonstrated by the following experiment: *Phycomyces blakesleeanus* was grown in the nutrient solution containing one part of thiamin in twenty million parts of the medium. Ten days later when the colony reached its maximum development, it was removed, washed with sterilized distilled water and transferred to a flask containing distilled water. One month later the mycelium was removed, washed in distilled water, transferred to a volume of distilled water and extracted by autoclaving. After the nutrients were added to this, sterilized and inoculated, it was found that *Phytophthora erythroseptica*, a thiamin-requiring organism, was induced to grow.

Bonner and Erickson (1) failed to obtain growth in thiamin-requiring *Phytophthora cinnamoni* when they added to their medium varying amounts of mycelium of *Phycomyces blakesleeanus* grown in a basic solution that contained a mixture of pyrimidine and thiazole. This appears to leave un-

answered the question of the ability of this species of Phycomyces to synthesize thiamin; yet table II shows that a number of thiamin-requiring fungi grew well in the presence of the mycelium extract of *Phycomyces blakesleeanus*. The cause of this discrepancy appears to lie in the Schopfer's medium used by BONNER and ERICKSON. While the abnormally high concentration of dextrose in Schopfer's solutions is readily utilized by a number of zygomycetous fungi, many of the Oomycetes, such as Phytophthoras, make no, or very little, growth in the presence of so much sugar. The small amount of thiamin present in the mycelium of *Phycomyces blakesleeanus* apparently was not enough to overcome the inimical effect of 10 per cent. dextrose.

Additional support to thiamin synthesis by Phycomyces is given by the following experiment: *P. blakesleeanus* was grown in the basic solution to which was added a mixture of pyrimidine and thiazole equivalent to 1/10th p.p.m. thiamin. Ten days later the mycelium was harvested, air dried, and varying amounts of it (at the rate of 1000, 250, 50, and 10 p.p.m.) were added to the basic medium. Twenty-five ml. of this nutrient solution was placed in a series of 250-ml. capacity flasks, sterilized and inoculated with *Phycomyces blakesleeanus* and *Phytophthora erythroseptica*. The organisms were incubated at 25° C. for 10 and 24 days respectively; the mycelium was then harvested and dried to constant weight at 100° C. Each figure in table III represents the aggregate weight of five cultures.

TABLE III

THE YIELD OF MYCELIUM INDUCED BY ADDING VARYING QUANTITIES OF DRIED MYCELIUM OF *Phycomyces blakesleeanus* TO THE MEDIUM

THE RATE OF DRIED MYCELIUM OF *P. blakesleeanus* ADDED TO THE BASIC MEDIUM	YIELD OF *P. blakesleeanus*. WEIGHT OF TOTAL MYCELIUM	YIELD OF *Ph. erythroseptica*. WEIGHT OF TOTAL MYCELIUM
p.p.m.	*mg.*	*mg.*
1000	211	325
250	199	273
50	113	271
10	72	28

The decrease in yield of mycelium, particularly in case of *Phytophthora erythroseptica*, is not proportionate. In a nutrient solution of low concentration, such as used in this work, the optimum amount of thiamin needed by this organism is extremely small and an amount of more than 1/1000th p.p.m. thiamin will exert little effect upon the yield. The significant fact brought out by the foregoing table is that *Phycomyces blakesleeanus* is capable of linking pyrimidine and thiazole to form thiamin as demonstrated by the fairly large yield of mycelium made by the thiamin-requiring *Phytophthora erythroseptica*.

Referring back to table II it should be noted that *Pythiomorpha gonapodioides*, unlike *Phycomyces blakesleeanus*, can synthesize its own thiazole which it links with pyrimidine to produce thiamin, retains the latter, and breaks down into its first disintegration products some of which escape into the medium.

Rhizopus suinus and *Fusarium niveum* are capable of synthesizing their own thiamin as well as biotin from pure dextrose, amino acids, and inorganic salts.

The case of *Mucor ramannianus* stands out as a glaring exception in the behavior of the other five fungi used in auxithal production. It is a uniformly poor source of thiamin and pyrimidine, the former being present in the hyphae in extremely minute amounts as shown by the behavior of *Phytophthora erythroseptica* and *Thielaviopsis basicola*, two organisms that react to mere traces (0.1 microgram per liter) of thiamin. The pyrimidine content also is very low in the mycelium, and extremely so in the medium and only the more sensitive pyrimidine-requiring organisms are capable of making a fair growth on its extracts. Both the mycelium and the medium of *Mucor ramannianus* contain thiazole, but not as much as those of the other organisms.

The inability of *Mucor ramannianus* to produce appreciable amounts of auxithals led the writers to believe that probably 1/20th p.p.m. thiazole used to produce the mycelium was not sufficient; or perhaps the fungus would do better if the pyrimidine were furnished extraneously; or if some thiamin were added to the thiazole. Consequently, the following experiments were made: a series of solutions was prepared by adding to the basic solution:

a. 1 p.p.m. thiazole
b. 1/20th p.p.m. thiazole and 1/20th p.p.m pyrimidine
c. 1/20th p.p.m. thiazole and 1/20th p.p.m. thiamin.
d. 1/20th p.p.m. thiamin

The foregoing solutions were placed in a series of Fernbach flasks (500 ml. per flask), sterilized, inoculated, incubated for 14 days and then the mycelium and the medium were treated as before; the extracts were added to the basic medium, solidified with agar, tubed, sterilized, inoculated, and incubated. Table IV shows the results.

Increasing the thiazole from 1/20 p.p.m. to 1 p.p.m. failed to bring about a significant increase in the auxithal production by *Mucor remannianus*. It appears that in the absence of an external supply of pyrimidine or thiamin the fungus synthesizes enough pyrimidine and thiamin for its immediate use, with very little left over. When, however, pyrimidine is added to the thiazole, the picture changes; the fungus synthesizes more thiamin than it needs, stores the bulk of it in the mycelium, and lets a trace of it diffuse into the medium. In addition to this, the pyrimidine content of both the mycelial

TABLE IV

THE EFFECT OF ADDING THIAMIN AND PYRIMIDINE TO THIAZOLE IN THE CULTURE MEDIUM UPON AUXITHAL PRODUCTION BY *Mucor ramannianus*

ORGANISMS	1 P.P.M. THIAZOLE		1/20 P.P.M. THIAZOLE 1/20 P.P.M. PYRIMIDINE		1/20 P.P.M. THIAZOLE 1/20 P.P.M. THIAMIN		1/20 P.P.M. THIAMIN	
	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT
Blakeslcea trispora	VIII	VI	X	VI	X	X	VIII	VI
Chaetocladium brefeldii	II	I	V	II	V	I	IV	I
Collybia tuberosa	II	I	VII	V	VII	II	VII	II
Cyathus striatus	IV	II	V	V	IV	IV	IV	IV
Deconica inquilina	II	I	V	III	IV	III	IV	III
Glococystidium roseo-cremeum	II	I	X	II	X	I	X	I
Lentinus tigrinus	X	II	X	X	X	X	X	X
Lenzites saepiaria	I	I	X	III	VIII	III	VIII	III
Lenzites betulina	IV	II	X	X	VIII	VII	VIII	V
Mucor ramannianus	X	X	X	X	X	X	X	V
Phycomyces blakesleeanus	III	I	X	VII	X	V	X	I
Phytophthora erythroseptica	II	I	X	III	X	III	X	II
Phytophthora fagopyri	V	I	X	X	X	V	X	V
Pilaira moreaui	IV	II	VIII	III	VIII	III	III	III
Pythiomorpha gonapodioides	V	I	X	V	X	V	VIII	V
Pythiomorpha oryzae	I	I	VIII	I	VIII	I	VIII	I
Pythium ascophallon	I	I	X	II	X	I	X	I
Pythium oligandrum	I	I	VII	IV	VII	I	VII	II
Pythium polymastum	I	0	VI	I	VII	II	VII	II
Thielaviopsis basicola	V	I	X	V	VII	III	VI	III
Typhula variabilis	VIII	I	X	VII	X	X	X	VIII

extract and the extract of the medium is increased, and a richer growth of all organisms is the result. As seen in the last two columns of the foregoing table, 1/20 p.p.m. thiamin alone is not as favorable as a mixture of 1/20 p.p.m. each of the two moieties because, perhaps, it contains proportionately less pyrimidine and thiazole.

The increase of auxithals paralleled a very rapid acceleration of growth when pyrimidine or thiamin was added to the thiazole. A more rapid growth, however, or a larger yield of mycelium does not necessarily mean a larger percentage of auxithals per given unit of mycelium. This was demonstrated by the following experiment: 0.05 per cent. of agar-agar was added to the basic medium containing 1/20 p.p.m. thiazole; this solution was placed in Fernbach flasks, sterilized, inoculated with *Mucor ramannianus* and incubated for 14 days. An increase of mycelium of about 50 per cent. followed the addition of agar-agar to the nutrient solution. This growth-accelerating property of agar is caused by the minor elements present in it. When the mycelium and the medium were treated as before, however, and tested, they failed to induce in the test organisms a better growth than did the mycelium formed in the agar-free solution.

The biotin content of the mycelium and the medium of variously treated cultures of *Mucor ramannianus* failed to show any significant increase. This would probably indicate that in so far as this organism is concerned, thiamin or its moieties do not exert a direct control on the biotin synthesis.

Summary

Six organisms were tested for their ability to synthesize biotin, thiamin, and the two moieties of thiamin. Two of these fungi, *Rhizopus suinus* and *Fusarium niveum*, are capable of synthesizing biotin and thiamin from pure dextrose, amino acids, and inorganic salts. *Phytophthora erythroseptica* is unable to grow without an external supply of thiamin but can synthesize biotin; *Phycomyces blakesleeanus*, if furnished with pyrimidine and thiazole will link them together and produce thiamin; *Pythiomorpha gonapodioides*, when supplied with pyrimidine, will elaborate its own thiazole, link it with pyrimidine, and form thiamin; *Mucor ramannianus* can synthesize thiamin if the medium contains thiazole, the fungus being able to produce its own pyrimidine. All three organisms, Phycomyces, Pythiomorpha, and Mucor can synthesize biotin, as shown by the growth of biotin-requiring fungi in the presence of the extract of the mycelium or the medium of these fungi.

Mucor ramannianus produces less biotin and thiamin than the other five fungi. When both thiazole and pyrimidine are added to its medium instead of thiazole alone, however, the resulting mycelium supports good growth in organisms that must have either thiamin or the moieties.

The bulk of thiamin is stored in the mycelium and only traces of it pass into the medium; appreciable quantities of pyrimidine and thiazole, how-

ever, diffuse into the nutrient solution. The same applies to biotin except that comparatively larger amounts of biotin find their way into the medium.

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MEASUREMENT OF THE RESPIRATORY QUOTIENT OF PLANT TISSUES IN A CONSTANT GASEOUS ENVIRONMENT

ALBERT ULRICH

(WITH THREE FIGURES)

Introduction

In the course of plant physiological investigations it is often necessary to determine the respiratory activity of living tissues. The rate of carbon dioxide evolution has been used frequently as an index of respiration since it is relatively easy to measure. Under some conditions, however, the carbon dioxide released does not reflect certain biochemical changes associated with respiration which may be more closely correlated with oxygen absorption. If both gases are measured simultaneously, and the respiratory quotient (CO_2/O_2) calculated, a better interpretation of attendant phenomena may be given than if an analysis is made of only one gaseous component of the system. For example, erroneous conclusions may be drawn concerning the influence of low oxygen tensions, and the effects of various absorbed salts on the formation of organic acids in excised barley roots when CO_2 measurements alone are used as a basis for measuring the respiratory processes.

Many methods for determining the respiratory quotients of living tissues have been proposed according to which samples of gas were withdrawn from an enclosed chamber and then subsequently analyzed for carbon dioxide and oxygen. Objections to these methods were based on changes in composition of the gaseous environment during the experimental period and in the necessity of great accuracy in the analytical estimations. Major improvements were developed by FERNANDEZ (**2**), who provided a closed system with a Bunsen valve pump for circulating the gases through alkali towers to absorb the carbon dioxide, and an oxygen generator controlled by an operator to replace electrolytically an oxygen deficit indicated by a manometer. His apparatus was adopted in a slightly modified manner by WETZEL (**7**) and more recently in revised form by BENNET-CLARK (**1**). The latter investigator introduced a non-pulsating pump and the automatic addition of oxygen.

Description of apparatus

The apparatus as originally designed by these workers has been simplified and changed in certain respects (fig. 1). The pump used (fig. 2) was a modification of that originally designed by BENNET-CLARK (**1**). Reiset absorption towers (D, fig. 1) as modified by STEWARD (**4**) were substituted for Pettenkofer tubes to absorb the carbon dioxide evolved from the plant tissues. The quantity of oxygen absorbed by the plant tissues was measured

by weighing the copper (or silver[1]) deposited on the cathode of the coulometer, O, connected in series with an oxygen generator, L (symbols refer to fig. 1). The volume, and therefore the composition of the circulating gas, were automatically maintained by means of a mercury manometer, K, which operated the entire electrical circuit. The fluctuations of the manometer resulting from the surging of the alkali in the absorption tower and from the slight pulsations of the pump were eliminated by connecting one arm of the manometer to the plant chamber, G. Uniform aeration of the submerged plant tissue was provided by matched sintered glass aerators F (3).

Construction and operation of pump

Details, drawn to scale, for the construction of the pump are shown in figure 2. Figure 2A is a longitudinal section and 2B and 2C are cross sections at K and M respectively. The pump shaft C is driven by a one-quarter horse power motor through the worm A, and gear B, at an approximate speed of 120 r.p.m. The $\frac{3}{4}$ inch steel shaft C, resting upon the ball bearing P, is held in a vertical position by brass bearings D and Q. D and Q are threaded to the brass cylinder F ($4\frac{3}{4}$ inches in diameter and $14\frac{3}{8}$ inches in length), the latter enclosing the three rollers K. The duralumin rollers ($\frac{3}{4} \times 2\frac{1}{4}$ inches) are hollow cylinders sliding on the rotating monel metal axels J ($\frac{3}{8} \times 4\frac{3}{4}$ inches), the rollers being held in place by the ridges L, and by the slots in M (fig. 2C). M is a brass spool held firmly in place by the pin through the shaft C. N and O are essentially truncated cones having a height of $1\frac{1}{8}$ inches and diameters of $3\frac{7}{8}$ and $1\frac{13}{16}$ inches. O is soldered to the shaft C, while N is movable but held in place by the spring G, and collar H. The collar V prevents the upward movement of the shaft when the pump is in motion. The slit I, in the cylinder F, facilitates the adjustment of the spring tension by changing the position of H to accommodate different sizes of rubber tubing S. Ordinary flexible black rubber tubing having a $\frac{1}{4}$-inch bore and a $\frac{1}{16}$-inch wall gave an air flow of 400 ml./min. R is a brass cylinder threaded to fit Q, thus forming a basin which holds the glycerin U, necessary to lubricate the rollers and rubber tubing.

To assemble the pump, the rubber tubing in the form of a loop is passed through T (a $\frac{1}{2} \times 1\frac{3}{8}$-inch hole in the brass tubing F) and then stretched tightly around the rollers of the shaft assembly (without F, D, and Q, and the gears) (fig. 2B). The cylinder F is slipped over the rubber tubing and set up as shown in fig. 2A. The flange on D serves to suspend the lower end of the pump R in the water bath after the cylinder F has been placed into a semi-circular notch cut into the board E. In order to replace the rubber tubing it is not necessary to take the pump apart, but to tie the ends of the

[1] In the present investigation it was found that Cu could be satisfactorily substituted for Ag in the coulometer. 5.68 mg. Cu = 1 ml. O_2 at standard conditions.

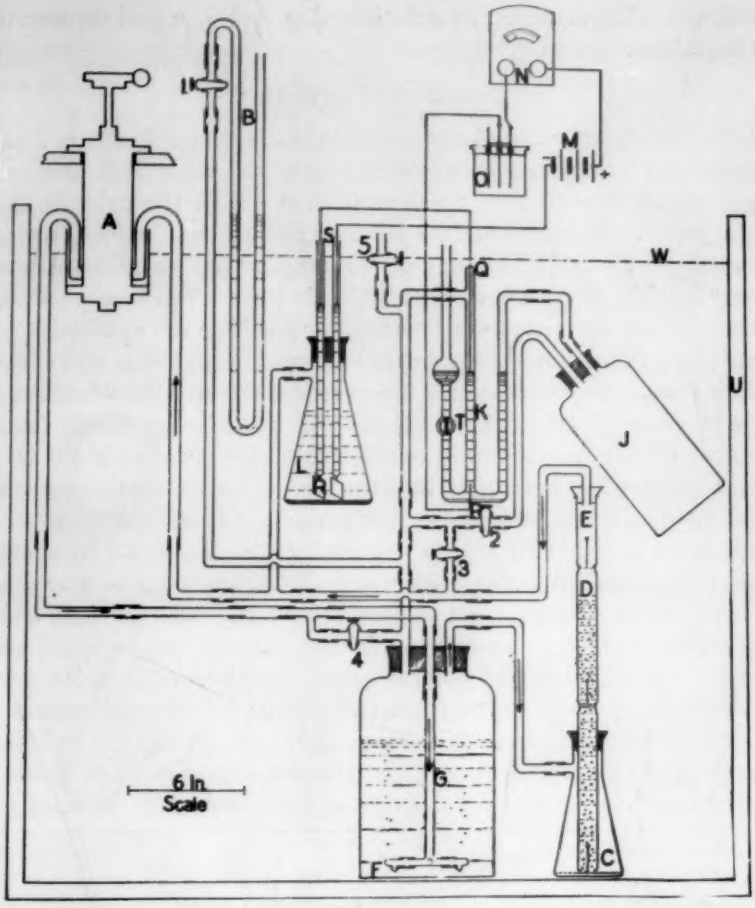

FIG. 1. Apparatus for determining the respiratory quotient of living tissues. The symbols designate the following:

A. Pump. See figure 2 and description in text.
B. Water manometer, 7-mm. glass tubing.
C.[2] 250-ml. Pyrex suction flask containing 100 ml. of NaOH.
D. Modified Reiset absorption tower. Pyrex glass tubing 2.5 cm. (outside diameter) × 43 cm.
E. Silver disk with perforations 0.022 inch in diameter.
F. Four matched sintered glass aerators.
G.[2] Four-liter wide-mouth jar fitted with rubber stopper.
J. Air reservoir, volume approximately equal to air space of circulating system.
K. Hg manometer constructed from 7-mm. glass tubing and filled with pure Hg.
L. O_2 generator, consisting of a 500-ml. suction flask containing $CuSO_4$ solution (150 gm. $CuSO_4 \cdot 5\ H_2O$ plus 1000 gm. H_2O) and solid $Cu(OH)_2$.

[2] The connections between G and C should be at an absolute minimum in order to avoid the escape of carbon dioxide through the rubber tubing.

old and new tubing together by a flexible wire, and then pull the new tubing over the rollers.

Operation of apparatus

After the apparatus has been assembled as indicated in figure 1 and the temperature of the system equalized to that of the water bath, short circuit the coulometer O to prevent the deposition of Cu on the weighed cathode, open stopcocks 1 to 5 to permit the pressure throughout the entire apparatus to become equal to the pressure of the atmosphere, and then adjust the mercury level in the arm Q of K so that it is just "off" as indicated by the ammeter N. After the mercury manometer has been once adjusted, it may be used many times, although it should be checked both before and after each determination. Next, disconnect the electrical circuit, add the plant material to G, close the stopcocks in the order of 5, 2, 3, and 4, and finally, if there is no change in pressure in the system as shown by the water levels in B, close number 1. Reconnect the electrical circuit so that copper is deposited upon a freshly prepared copper cathode in O and start the pump A. At this point it would be well to mention that if stopcock 1 is allowed to remain open when the pump is in operation, there should be a very slight positive pressure in the system as recorded by B. This pressure causes a slight depression of the mercury manometer, but as soon as a small amount of oxygen is absorbed by the plant material, the mercury in K is drawn up to the point on Q to close the circuit, and oxygen is generated within a few seconds to restore the pressure in the system. At the end of the run, stop the motor, open the electrical circuit at O, open stopcocks 3 and 4, and then close the circuit again at O. If, as previously stated, the pressure on the

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M. 6-volt storage battery.

N. Ammeter.

O. Coulometer (voltameter). The center cathode is of electrolytic copper,[3] 0.008 inch thick and about 20 cm.^{2} in area. The anodes are of heavy pure Cu. The electrolytic solution is prepared by adding 150 gm. of $CuSO_4 \cdot 5H_2O$, 50 gm. concentrated H_2SO_4, and 50 gm. 95 per cent. alcohol to 1000 gm. of water.

P and Q. Tungsten wires. Q is sharpened to a fine point by fusing with $NaNO_2$.

R. Pt plates 2–3 cm.^{2} in area.

S. 7-mm. glass tubes filled with Hg.

T. Ground glass stopcock.

U. Constant temperature bath.

W. Water line.

Numbers 1, 2, 3, 4, and 5 are ground glass stopcocks.

[3] The Cu electrodes were prepared by submerging them in a vessel containing 1:1 nitric acid. When the surfaces were clean, distilled water was added and allowed to overflow the vessel until the acid became very dilute. The plates were removed and thoroughly washed with distilled water, followed by a small amount of 95 per cent. alcohol. The electrodes were dried over a sand bath and placed in a desiccator before weighing.

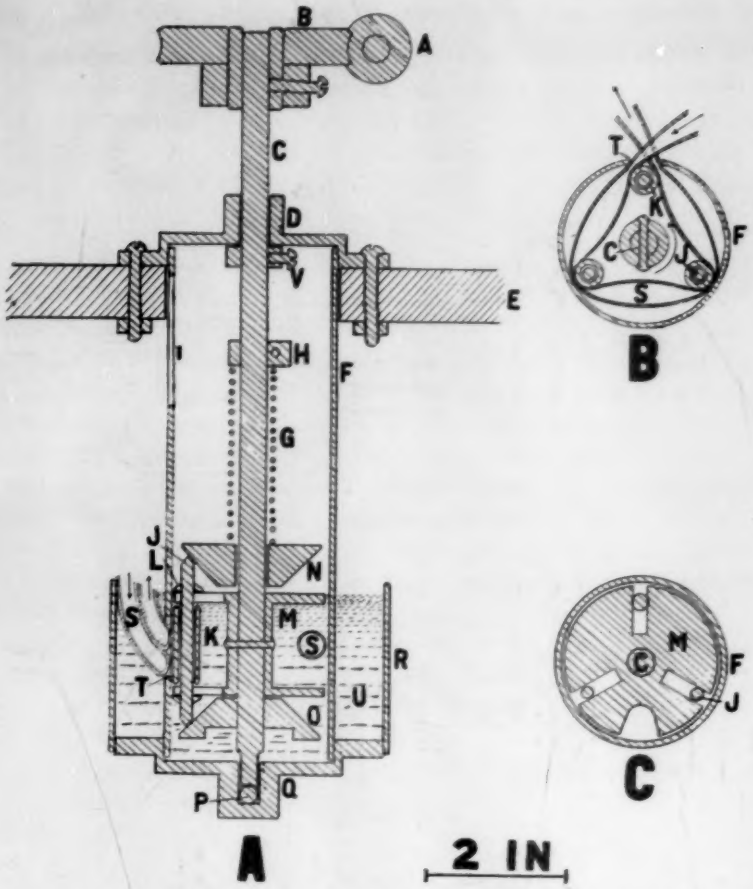

FIG. 2. Longitudinal section of pump (A) and cross sections (B) and (C) at K and M respectively. See text for explanation of symbols.

mercury manometer has been slightly positive, then a very slight reduction in the total volume of the system is necessary before oxygen can be generated to replace that absorbed by the plant material. In order to restore the original volume in the system, oxygen is generated for approximately 15 seconds until the mercury manometer is again just "off." (A negative pressure on the mercury manometer at the start cannot be readily compensated for at the end of the experiment. A negative or positive pressure can usually be obtained by changing the resistance of the sintered glass aerators, F). When the equilibrium has been restored, weigh the copper cathode, remove the tower containing alkali (usually 0.1 to 0.5 N NaOH) and determine the CO_2 evolved by WINKLER'S method (6).

Oxygen tensions

The oxygen tension in the circulating system may be changed as desired by introducing a gas of known composition into the apparatus at the start of the experimental period. After placing the plant material into chamber G, a convenient procedure is to force the gaseous mixture (oxygen and nitrogen) from the storage cylinder X through the apparatus by pump A until the air has been completely displaced. The schematic drawing (fig. 3,

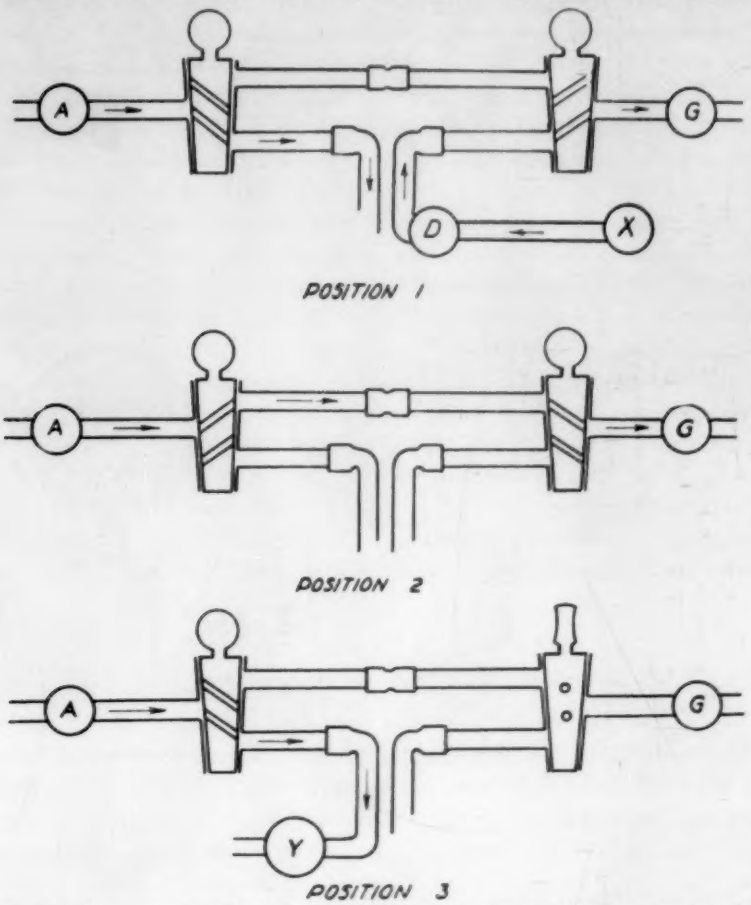

Fig. 3. Arrangement of equipment for introducing various oxygen tensions into the apparatus. A, pump; D, Reiset absorption tower; G, plant chamber; X, gas cylinder; Y, glass bulb.

position 1) indicates the arrangement of the necessary equipment. After the apparatus including the by-passes has been aerated sufficiently, the pump and gas supply are turned off, the stopcocks turned to position 2, and

thereafter the procedure is the same as outlined in the operation of the apparatus. If, at the end of the experimental period, a sample of the enclosed gas is desired for analysis, it may be withdrawn by turning the stopcocks to position 3, and then pumping the gas into a glass bulb Y, using the technique ordinarily adopted for gas analyses.

Calculations

One of the distinct advantages of the system just outlined is the ease with which the results are calculated. In those procedures which depend upon observed pressure changes in order to determine the oxygen absorbed in a closed system, numerous corrections must be made for fluctuations in atmospheric pressure while operating the apparatus. At the same time there must also be considered the usual temperature, pressure and volume relationships of gases under varying experimental conditions. These troublesome calculations are entirely eliminated when once the electro-chemical equivalence between the oxygen generated and the deposited copper is ascertained. This simplification is due to the fact that the oxygen absorbed by the plant material is returned to the closed system under exactly the same conditions as those under which it was removed and consequently the weight of the copper deposited in the coulometer is directly related to the oxygen required to restore the equilibrium. The determination of the carbon dioxide evolved offers no difficulties in this or most other systems (1 ml. of 0.1 N NaOH neutralized is equivalent to 1.113 ml. CO_2 under standard conditions).

The electrolytic reactions which occur in the oxygen generator containing a solution of copper sulphate and solid copper hydroxide are as follows:

Ionization of water:

$$H_2O \rightleftarrows H^+ + OH^-$$

In the cathode compartment:

$$2H^+ + Cu(OH)_2 \rightarrow Cu^{++} + 2\,H_2O$$

$$Cu^{++} + 2E \rightarrow Cu \text{ (deposited on cathode)}$$

In the anode compartment:

$$2\,OH^- \rightarrow H_2O + \tfrac{1}{2}\,O_2 \text{ (evolved)} + 2E$$

Normally in electrolysis the hydrogen ions which are attracted toward the cathode form hydrogen gas, but in the presence of an excess of $Cu(OH)_2$, they are neutralized by the OH ions and copper is deposited instead. Under these conditions four equivalents of copper (127.14 gm.) are deposited for each mole of oxygen generated (22,400 ml. at 760 mm. and 0° C.). Accordingly, one cubic centimeter of oxygen is equivalent to 5.68 mg. of copper.[4]

[4] In a recent communication from Dr. F. C. Steward, the formation of oxidation products other than oxygen, particularly ozone, was noted in small quantities during the

Test runs

The apparatus was tested in several ways in order to determine the efficiency of the carbon dioxide absorption and the extent of the replacement of the gas by oxygen. The first series of blank determinations were designed to ascertain the recovery of carbon dioxide only. Known amounts of solid C.P. potassium bicarbonate (Merck's blue label) were introduced into a 300-ml. Erlenmeyer flask just ahead of the chamber G (fig. 1). This was followed by a small crucible containing approximately 5 ml. of 1:1 H_2SO_4. After the entire apparatus has been swept free of carbon dioxide and the absorption tower replaced with one containing fresh alkali, the flask was shaken vigorously, spilling the acid onto the potassium bicarbonate. The carbon dioxide released is recorded in table I.

TABLE I

Carbon dioxide recovery from potassium bicarbonate

Number	CO_2 as $KHCO_3$ added	CO_2 recovered	Recovery
	ml.	*ml.*	%
21	44.8	45.7	102.0
22	44.8	44.7	99.8
31	44.8	44.8	100.0
33	44.8	44.8	100.0
23	67.2	66.1	98.4
24	67.2	66.2	98.5

Since the carbon dioxide recovery was satisfactory, a second test was undertaken in which the flask of the previous series was replaced by a glass bulb having a stopcock at each end. When this bulb was filled with carbon dioxide at the prevailing atmospheric pressure and when its temperature was that of the apparatus, it was connected into the circulating system just ahead of the respiratory chamber G. The carbon dioxide which was absorbed by the alkali scrubber was replaced by generated oxygen. The results from several determinations using the two bulb sizes are given in table II.

Typical results from excised barley roots taken from plants grown under standard conditions and subsequently subjected to a uniform treatment are given in table III. The fluctuations noted in the table were due to the variations in the root material itself and to the errors in measurement of respiration. A better criterion for evaluating the apparatus would have been through the use of absolutely uniform plant material.

generation of oxygen. Through the use of Schiff's nitrometer, the average quantity of copper deposited per ml. of oxygen (at 760 mm. and 0° C.) was found in this laboratory to be 5.75 ± 0.0071 mg. The figure 0.0071 is the standard error of the mean of 16 separate determinations. The difference of 0.07 mg. (or 1.2%) from the calculated value of 5.68 mg., while significant, is within the limits of error of the oxygen determination with the described apparatus and well within the variation of most biological material.

TABLE II

REPLACEMENT OF ABSORBED CARBON DIOXIDE BY GENERATED OXYGEN

NUMBER	SIZE OF BULB	CO_2 ABSORBED	O_2 GENERATED	CO_2/O_2
		ml.	*ml.*	
1	Small	50.80	50.14	1.013
2	Small	50.18	50.39	0.996
3	Large	67.14	63.91	1.051
4	Large	66.03	65.49	1.008
5	Large	66.56	66.14	1.006

TABLE III

RESPIRATORY ACTIVITY OF 100 GRAMS OF COMPARABLE EXCISED BARLEY ROOTS

NUMBER	CO_2 PRODUCED	O_2 ABSORBED	R.Q. CO_2/O_2
	ml.	*ml.*	
422	174.5	180.5	0.967
426	171.6	183.1	0.937
566	172.4	190.5	0.905
608	180.6	193.2	0.935
732	172.7	180.3	0.958
752	168.5	189.5	0.889

Summary

A description is given of a simplified apparatus for determining simultaneously the carbon dioxide evolved and the oxygen absorbed by plant tissues under a constant gaseous environment: (1), to permit the introduction of Reiset absorption towers for absorbing carbon dioxide; and (2), oxygen absorbed by the plant tissues was replaced electrolytically by a generator operated automatically by a mercury manometer. The quantity of current passing through the generator was measured by a coulometer in series. This method was successfully applied to a study of respiration in excised roots for the purpose of gaining further information on organic acid metabolism as related to the absorption of cations and anions (**5**).

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MICRO DETERMINATION OF SOME CONSTITUENTS OF PLANT ASH[1]

MONROE E. WALL

Introduction

Macro-gravimetric or volumetric procedures involved in the determination of the elements found in plant ash require a large sample and many of the operations are time consuming and tedious. Micro determinations, on the other hand, are much more rapid, require much less tissue, and are convenient to use. If very small quantities are involved, these micro methods are also more accurate; macro methods will give better results, however, if a large enough sample is obtainable.

In many investigations, comparative results are the primary objective. The ash absorption of plants grown at various potassium levels is an example. Here the use of more convenient and rapid micro methods is permissible. On the other hand, there are many instances where the amount of sample available is so small, that micro methods are not only accurate but also necessary tools.

The object of this paper is to describe certain micro methods used in determinations for constituents of plant ash. The procedures described are mostly colorimetric, involving the use of a simple, inexpensive, photoelectric colorimeter. With suitable standards, the visual colorimeter, or spectrometer, may be used in place of the photoelectric colorimeter.

Methods and results

SOLUBLE MINERALS

One-half to one gram of dry, finely pulverized plant material is placed in a 250-ml. wide mouthed flask. One hundred ml. of distilled water are added and the suspension is refluxed for one hour. The residue is filtered off by suction and washed thoroughly with hot water. A negative or faintly positive diphenylamine test for nitrates on Nessler's test for ammonia on a drop of the washings are good criteria for complete extraction of soluble minerals.

The filtrate is evaporated to approximately 50 ml. and then transferred to a 100-ml. volumetric flask and made up to volume. A few drops of chloroform and toluene are added and the flasks are kept in a cool place. Sodium, potassium, calcium, magnesium, and sulphates can be determined as in total ash analyses. Nitrates may be determined on a decolorized

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Vegetable Gardening. This investigation was conducted under a Fellowship Grant from the American Potash Institute.

aliquot (**5**). To determine phosphates, a 5-ml. aliquot is placed in a 50-ml. beaker, and approximately 0.1 gm. of Darco charcoal is added and stirred for one to two minutes. The material is then filtered into a 100-ml. volumetric flask and the filter washed thoroughly. The determination is then made as for total ash.

Total ash

One-half to one gram of dry, finely pulverized plant material is ashed in a muffle at 600° C. for one hour. The ash is weighed if desired, and then taken up in 50 ml. of 1:4 hydrochloric acid. Most of the nitrates are lost in ashing by this procedure, and hence nitrates must be determined on the aqueous extract. Plants grown on soil should have the silica removed at this point by evaporating the ash solution to dryness and heating for one hour on a water bath. The residue is moistened with 5 to 10-ml. concentrated HCl, and 25 ml. of water are added. The solution is heated for a few minutes on a warm water bath, and is then filtered through a hard filter paper and washed thoroughly. The filtrate is evaporated to approximately 25 ml. to drive off most of the HCl and is then made to volume in a 100-ml. volumetric flask (**1**).

This procedure is not necessary for plants grown in sand or water culture since the silica content is low. In this case the ash solution is evaporated to 25 ml. and made to volume in a 100-ml. volumetric flask.

Colorimetric determination of phosphates

Principle.—When phosphates react with the molybdate ion a phosphomolybdenum complex is formed. This compound when reduced will give a blue color (**3**). Arsenates and silicates will interfere. Arsenates are not found to any appreciable extent in plant ash. Silicates are also low in plants grown in sand and water culture. Silicates may be removed if necessary by appropriate technique (**1**).

Reagents.—Five per cent. ammonium molybdate in $2N-H_2SO_4$, 1 per cent. hydroquinone in $2N-H_2SO_4$, and 16.5 per cent. sodium bisulphite.

Standard.—KH_2PO_4—0.4380 gm. in one liter; 1 ml. = 0.1 mg. phosphorus.

Procedure.—A 5-ml. aliquot of ash solution is pipetted into a 100-ml. volumetric flask. Four ml. of ammonium molybdate, 2 ml. of hydroquinone, and 4 ml. of sodium bisulphite solution are added. The flask is shaken, and the solution made to volume, stoppered, and set aside for one-half hour. It is convenient to run six samples in duplicate, spacing each set of duplicates 5 minutes apart. At the end of one-half hour, the colorimeter readings are taken. The standard curve is prepared in the same manner, except that aliquots can be any volume desired.

Results on known phosphate samples.—In this and other discussions of the accuracy of the methods presented, it will be assumed that the

knowns are present in the same amount of total sample as the unknowns. For instance, if 0.500 gm. plant material is ashed, dissolved, and made up to a volume of 100 ml., then a 5-ml. aliquot (the quantity used in the phosphate determination) would correspond to 25 mg. The percentage of known phosphate is calculated using this quantity as a basis of estimation.

Theoretical	Found	Absolute error	Relative error
%	%	%	%
0.480	0.492	+ 0.012	2.5
0.560	0.584	+ 0.024	4.3
1.000	0.980	− 0.020	2.0
1.120	1.080	− 0.040	3.6
1.440	1.460	+ 0.020	1.4
1.680	1.700	+ 0.020	1.2
2.200	2.128	− 0.072	3.3
3.000	3.000	0.000	0.0
3.520	3.540	+ 0.020	0.6
			—
		Average	2.0

The determination of magnesium

Principle.—Magnesium is separated from calcium as mentioned later. (*Cf.* p. 544.) The magnesium is precipitated as $MgNH_4PO_4$ (**10**), and the magnesium estimated from the phosphate content.

Reagents.—Two per cent. $NH_4H_2PO_4$, concentrated NH_4OH, and four per cent. NH_4OH.

Standard.—$Mg(NO_3)_2 \cdot 6\ H_2O$—10.538 gm. in one liter; 1 ml. = 1 mg. magnesium. Dilute if necessary.

Procedure.—The supernatant washings from the calcium determination are decanted into a 50-ml. beaker, and evaporated until salts begin to crystallize out (usually 2 to 3 ml. volume). The solution is then quantitatively transferred to a 15-ml. centrifuge tube, using a small funnel. The beaker is washed three times, using not more than 2 ml. of water each time. The centrifuge tube is then placed in a boiling water bath and 2 ml. $NH_4H_2PO_4$ are added. Two ml. concentrated NH_4OH are added and stirred with a small rod. The rod is rinsed and the centrifuge tube is corked and allowed to stand over night. The precipitate of $MgNH_4PO_4$ is centrifuged and washed as discussed under calcium, the washings being discarded. The precipitate is dissolved in 2 ml. N H_2SO_4 and the resultant solution washed into a 100-ml. volumetric flask. Proceed as outlined under phosphates. The concentration of magnesium can be found by multiplying the mg. of phosphorous by 0.784.

The standard curve should preferably be made from a magnesium standard which is converted to $MgNH_4PO_4$. Good results were secured

using the phosphate standard if a blank of 0.2 mg. was subtracted from the final result. This blank was quite constant, whereas a series of magnesium knowns was consistently high by this value.

The following are results for magnesium in a known calcium-magnesium mixture:

Theoretical	Found	Absolute error	Relative error
%	%	%	%
0.250	0.320	+0.070	28.0
0.500	0.540	+0.040	8.0
0.750	0.680	−0.070	9.0
1.000	1.010	+0.010	1.0
1.250	1.240	−0.010	0.8
1.500	1.470	−0.030	2.0

Pure magnesium samples in the range corresponding to the first three results were accurate to 2–4 per cent. The difficulty seems to be in separating the small amount of magnesium from calcium. Hence the sample taken should have at least 1 per cent. magnesium; *i.e.*, 0.1 mg. magnesium in a 2-ml. aliquot of plant ash equivalent to 10 mg. of original sample.

Determination of potassium

Principle.—Potassium is precipitated as the sodium potassium cobaltinitrite. The precipitate is decomposed and the nitrite determined by the sulphanilic acid-naphthylamine reaction. The composition of the precipitate varies according to the conditions, but is constant if the conditions are constant (**11**). Ammonium, but not sodium will interfere.

Reagents.—Thirty per cent. sodium cobaltinitrite, 10 per cent sodium chloride, 0.1 N NaOH (approx.)

Naphthylamine-sulphanilic acid solution:

> 7.5 gm. sulphanilic acid in 750 ml. of 10 per cent. acetic acid mixed with 7.5 gm. naphthylamine in 750 ml. of 10 per cent. acetic acid. The naphthylamine is boiled in 200 ml. of water and then 10 per cent. acetic acid is added to make 750 ml. The reagent is kept in a dark bottle well stoppered. It should be only slightly pink. If necessary the solution can be decolorized by adding activated carbon and zinc dust, heating, and filtering.

Standard.—KCl: 0.382 gm. per liter, 1 ml. = 0.2 mg. potassium.

Procedure.—A 5-ml. aliquot of plant ash is pipetted into a 50-ml. beaker and evaporated to dryness on the steam bath. If a plant extract is used, the aliquot must be made alkaline with NaOH to drive off any ammonia present. The material is then acidified with HCl and evaporated as above.

One ml. H_2O and 2 ml. sodium cobaltinitrite reagent are added to the residue; the beaker is placed in the ice-box overnight. The precipitate is filtered through a Gooch crucible with a thin asbestos mat. The beaker and crucible are then washed with ice-cold 10 per cent. NaCl solution until the washings are colorless. The mat is picked out with a pointed rod and transferred to a 150-ml. beaker. Twenty ml. 0.1 N NaOH are added, washing the Gooch with the NaOH as it is added, the suspension is then brought to boiling. At this point the beaker should be rotated to bring all the asbestos into a uniform suspension. The decomposed cobaltinitrite precipitate is then filtered through a Gooch using a suction test-tube to catch the filtrate. The Gooch is washed well with hot water. The filtrate should be colorless or only slightly yellow.

ALTERNATIVE PROCEDURE.—One ml. plant ash is pipetted into a centrifuge tube, neutralized with NaOH and made slightly acid with HCl. Two ml. of sodium cobaltinitrite reagent are added and permitted to stand overnight in the ice-box. The precipitate is centrifuged 15 minutes at 2000–2500 r.p.m. and the supernatant liquid decanted. It is washed with 3-ml. portions of ice-cold 10 per cent. NaCl until washings are colorless, centrifuged and decanted each time as above. The precipitate is decomposed with 0.1 N NaOH, and need not be filtered.

This procedure is more accurate than the previous procedure, but the high speed and length of centrifuging make it inconvenient when many samples are to be determined.

The solution containing the decomposed cobaltinitrite precipitate is transferred to a 100-ml. volumetric flask and made to volume. A 10-ml. aliquot which should not contain more than 0.04 mg. potassium is pipetted into another 100-ml. volumetric flask, and 5 ml. naphthylamine-sulphanilic acid solution is added. The solution is made to volume with 10 per cent. acetic acid and allowed to stand 5 minutes before the microammeter reading is determined.

PREPARATION OF THE STANDARD CURVE.—One and one-half grams of $NaNO_2$ are dissolved and made up to 1 liter. Twenty ml. of this solution are made to 1 liter and used to prepare the standard curve. Aliquots are transferred to 100-ml. volumetric flasks, and the color is produced and determined as above. A known potassium solution is treated in the same manner as the unknowns, and the colorimeter reading determined. Hence the nitrite curve is standardized in terms of the nitrite reading of a known potassium solution.

REMARKS.—The necessity of greatly diluting the filtrate from the decomposed cobaltinitrite precipitate results in a large dilution error. If the filtrate is not diluted, precipitation of the diazo complex occurs. In addition the pink color produced in the final estimation does not give a favorable

RESULTS OF SOME KNOWN POTASSIUM DETERMINATIONS

THEORETICAL	FOUND	ABSOLUTE ERROR	RELATIVE ERROR
mg.	*mg.*	*mg.*	%
0.00200	0.00192	− 0.00008	4.0
0.00400	0.00384	− 0.00016	4.0
0.00600	0.00672	+ 0.00072	12.0
0.00800	0.00768	− 0.00032	4.0
0.01000	0.01090	+ 0.00090	9.0
0.01200	0.01184	− 0.00016	1.4
0.02000	0.02240	+ 0.00240	12.0
0.03200	0.03360	+ 0.00160	5.0
0.04000	0.04060	+ 0.00060	1.5
			Average 5.9

curve and a small error in reading may result in a large final error. When small quantities of potassium are to be determined, however, the method is more accurate than the $KMnO_4$ titration procedure.

DETERMINATION OF SODIUM

PRINCIPLE.—Sodium is precipitated as the uranyl zinc acetate $3UO_2(C_2H_3O_2)_2 \cdot Zn(C_2H_3O_2)_2 \cdot NaC_2H_3O_2 \cdot 9H_2O$. The uranyl radical is then converted into uranyl potassium ferrocyanide, $UO_2K_2Fe(CN)_6$ (**2**). Phosphate interferes and must be removed. Calcium, magnesium, strontium, barium, iron, and potassium do not interfere.

Phosphates have been removed by hydrated lime or zinc acetate, but these methods were not so satisfactory as precipitation of the phosphate as $MgNH_4PO_4$.

REAGENTS.—Zinc uranyl acetate (**9**), 10 gm. uranyl acetate in 50 ml. boiling water containing 2 ml. glacial acetic acid, and 30 gm. zinc acetate in 50 ml. boiling water containing 1 ml. glacial acetic acid.

Mix the boiling solutions and heat again to boiling and let stand overnight. Filter and mix the filtrate with an equal volume of absolute alcohol. Let stand forty-eight hours at 0° C. and filter at 0°. The reagent is stable at room temperature.

$Mg(NO_3)_2$ – 13 gm. per 1 liter

Concentrated NH_4OH

95 per cent. alcohol

20 per cent. potassium ferrocyanide

STANDARD.—0.1271 gm. NaCl in 1 liter; 1 ml. = 0.05 mg. sodium.

PROCEDURE.—Pipette 2 ml. of ash solution into a 15-ml. centrifuge tube. Add 2 ml. $Mg(NO_3)_2$ solution and place in a water bath and heat to 100° C.

and then add 2 ml. concentrated NH_4OH, cork the tube, and let stand overnight. Centrifuge at 1200 r.p.m. for five minutes. Pipette a 2-ml. aliquot into another centrifuge tube, taking care not to stir up the precipitate of $MgNH_4PO_4$. Heat the centrifuge tube to 100° C. in a water bath for fifteen to twenty minutes. This drives off most of the excess ammonia. Acidify with 1 or 2 drops glacial acetic acid. Add 4 ml. of zinc uranyl acetate reagent and stir until precipitation begins. Let stand one hour. Centrifuge at 1200 r.p.m. for five minutes, decant, and drain on filter paper, wiping the mouth of the centrifuge tube. Wash twice with 5 ml. of 95 per cent. alcohol, centrifuging and draining as before. Dissolve the precipitate in water and transfer to a 100-ml. volumetric flask. Add four drops acetic acid and 2 ml. potassium ferrocyanide reagent. Make to volume and let stand 3 minutes. Take the reading at once.

When the alcoholic uranyl zinc acetate is added to the unknown, the alcohol may precipitate some ammonium and magnesium salts. These do not affect the determination, and are easily soluble in water.

RESULTS ON KNOWN SODIUM DETERMINATIONS

THEORETICAL	FOUND	ABSOLUTE ERROR	RELATIVE ERROR
%	%	%	%
0.300	0.300	0.000	0.0
0.400	0.410	+0.010	2.5
0.500	0.500	0.000	0.0
0.550	0.530	−0.020	3.6
0.600	0.610	+0.010	1.7
0.650	0.640	−0.010	1.5
0.750	0.780	+0.030	4.0
0.900	0.860	−0.040	4.4
1.100	1.040	−0.060	5.5
			Average 3.3

VOLUMETRIC DETERMINATION OF CALCIUM

PRINCIPLE.—Calcium is precipitated as the oxalate under such conditions that the precipitation of magnesium is prevented. The precipitate of calcium oxalate is dissolved in H_2SO_4 and titrated with standard permanganate. The procedure is essentially that of KRAMER and TISDALL (**8**).

The calcium in the dissolved calcium oxalate can also be determined colorimetrically by precipitating as the phosphate, and then determining the phosphate ion colorimetrically. This procedure is more laborious.

REAGENTS.—Four per cent. ammonium oxalate, 4 per cent. ammonium hydroxide, 30 per cent. sodium hydroxide, 1:15 hydrochloric acid, 0.1 N $KMnO_4$ and 0.01 N $KMnO_4$.

STANDARD.—$Ca(NO_3)_2 \cdot 4H_2O$—5.9016 gm. per liter; 1 ml. = 1 mg. calcium. The standard may be diluted if necessary.

PROCEDURE.—Two ml. of ash solution are pipetted into a conical, 15-ml. centrifuge tube. One drop of methyl red solution is added, and 30 per cent. NaOH added drop by drop until the indicator turns yellow. (It is best to stir with a thin glass rod during this and any other operation involving the addition of reagents to this type of centrifuge tube, since diffusion is very slow. The rod can be washed with a thin stream of liquid from a hypodermic needle. This will keep the volume down.) One to fifteen HCl is added until the indicator turns barely pink. Two ml. of ammonium oxalate are added, the solution stirred, and the precipitate of calcium oxalate allowed to stand overnight. It is not essential to allow the precipitate to stand so long, but this procedure is convenient when one is working with a large number of samples.

The solution is centrifuged five minutes at 1200 r.p.m., and the supernatant liquid is decanted and saved for the magnesium determination. The precipitate is washed twice with 5 ml. of 4 per cent. NH_4OH, and is centrifuged and decanted each time as above, adding the supernatant liquid to the material for the magnesium determination. The calcium oxalate precipitate is dissolved in 2 ml. of N H_2SO_4, the centrifuge tube being heated on a boiling water bath. The resultant oxalate solution is titrated to a faint pink end point with 0.01 N $KMnO_4$, using a micro burette. In the titration it is best to keep the centrifuge tube immersed in a beaker of water at a temperature of 60 to 90° C. A blank determination should be run on the reagents and subtracted from the final result.

The 0.01 N $KMnO_4$ is made by diluting accurately standardized 0.1 N $KMnO_4$. It is used only for one day. The normality of the 0.1 N $KMnO_4$ should be checked every three to four days. The use of ceric ion with the

RESULTS OF THE DETERMINATION OF CALCIUM IN A KNOWN CALCIUM-MAGNESIUM MIXTURE

THEORETICAL	FOUND	ABSOLUTE ERROR	RELATIVE ERROR
%	%	%	%
0.250	0.220	−0.03	12.0
0.500	0.490	−0.01	2.0
0.750	0.760	+0.01	1.4
0.800	0.810	+0.01	1.3
1.000	1.030	+0.03	3.0
1.200	1.260	+0.06	5.0
1.500	1.470	−0.03	2.0
			Average 3.9

phenanthroline Fe++ indicator as a standard oxidant would obviate the necessity of frequent restandardizations since this reagent is quite stable.

Summary

1. The preparation of plant samples for mineral analyses has been described.

2. Colorimetric determinations of phosphate, magnesium, potassium, and sodium are presented together with data on the accuracy of the methods.

3. A micro volumetric determination of calcium is described and data on the accuracy of the method presented.

The author wishes to thank DR. V. A. TIEDJENS and PROFESSOR L. G. SCHERMERHORN for offering laboratory facilities and advice in carrying out this investigation.

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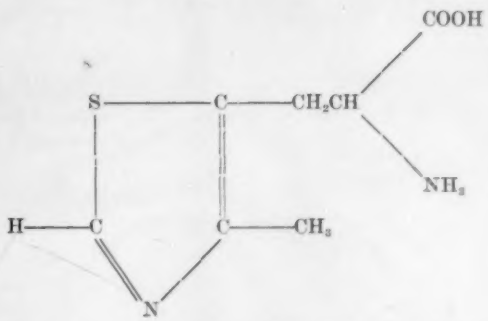
RESPONSE OF EXCISED TOMATO ROOTS TO β (–4–METHYLTHIAZOLYL–5) –ALANINE[1]

WILLIAM J. ROBBINS

(WITH ONE FIGURE)

The probability that thiamin (vitamin B_1) is essential for most if not all organisms makes any information on its synthesis *in vivo* of considerable importance. BUCHMAN and RICHARDSON (**2**) synthesized an amino acid, β (–4–methylthiazolyl–5)–alanine which they consider might be the precursor of the thiazole which makes an integral part of the thiamin molecule. The compound they synthesized has the following structure.



It differs from the vitamin thiazole in having a CH_2CHNH_2COOH group in place of the CH_2CH_2OH group of the latter compound. They state that the evidence accumulated to date does not permit definite conclusions regarding the biological rôle of the amino acid. BONNER and BUCHMAN (**1**), however, report pea roots able to convert the substance to the vitamin thiazole, and BUCHMAN and RICHARDSON (**2**) state that BONNER found *Phycomyces blakesleeanus* unable to bring about appreciable transformation and that KNIGHT found *Staphylococcus aureus* also unable to utilize it appreciably.

Since the strain of tomato roots cultivated in this laboratory for some years under sterile conditions is apparently able to grow indefinitely in a mineral solution containing sugar and the thiamin thiazole (**3**) it appeared worth while to determine its response to BUCHMAN'S compound. A sample of the β (–4–methylthiazolyl–5)–alanine was kindly supplied by BUCHMAN.

Excised tomato roots were grown in a solution containing per liter 0.333 gm. $Ca(NO_3)_2 \cdot 4H_2O$, 0.083 gm. KH_2PO_4, 0.083 gm. KNO_3, 0.041 gm. KCl, 0.083 gm. $MgSO_4 \cdot 7H_2O$ and 0.05 p.p.m. B, 0.05 p.p.m. Mn, 0.005 p.p.m.

[1] Assistance in this work was furnished by the personnel of Works Projects Administration Official Project 65-1-97-23. W. P. no. 5.

Zn. 0.002 p.p.pm. Cu, and 0.005 p.p.m. Mo. The root fragments were grown in 50 ml. of solution in 125–ml. Erlenmeyer flasks of pyrex glass at approximately 25° C. in weak diffuse daylight. The following solutions were used: 1, the mineral solution containing 2 per cent. Pfanstiehl C. P. cane sugar; 2, the same plus per flask 10 gamma of the amino acid; 3, plus 10 gamma of the amino acid; 4, plus 10 gamma of the thiamin thiazole; 5, plus 10 gamma of the amino acid and 10 gamma of the thiamin pyrimidine; 6, plus 30 gamma of the amino acid. All solutions were sterilized at 15 pounds pressure but the amino acid was filtered sterile and added to solutions 2, 5, and 6 after the other constituents had been sterilized by heat. The amino acid in solution 3 was autoclaved along with the balance of the medium. Each of the six solutions was prepared with 5 replications and inoculated with fragments of tomato roots which had grown 24 passages during a period of 2 years in a mineral solution containing sugar and thiazole.

When examined some weeks after inoculation there was no growth in the solution containing mineral salts and cane sugar but considerable in solutions 2, 3, 4, 5, and 6. The growth in the solution 2 with the heated

TABLE I

GROWTH OF EXCISED TOMATO ROOTS THROUGH THREE SUCCESSIVE PASSAGES IN THE SOLUTIONS NOTED

ADDITION TO MINERAL AND SUGAR SOLUTION	PASSAGE I MAY 4–JULY 13		PASSAGE II JUNE 28–SEPT. 1		PASSAGE III SEPT. 1–NOV. 15		RANGE DRY WEIGHTS	AVERAGE DRY WEIGHT PER ROOT FOR 3 PASSAGES
	NUMBER OF ROOTS	DRY WEIGHT PER ROOT	NUMBER OF ROOTS	DRY WEIGHT PER ROOT	NUMBER OF ROOTS	DRY WEIGHT PER ROOT		
		mg.		*mg.*		*mg.*	*mg.*	*mg.*
None	2	0.6	5	1.7	5	0.2	0.1– 0.4	0.8
10 gamma (65 μ) heated amino acid	3	3.7	5	6.6	5	5.1	0.8– 9.4	5.1
10 gamma (65 μ) filtered amino acid	4	2.3	5	1.7	5	3.6	2.7– 4.3	2.5
10 gamma (68 μ) thiamin thiazole	3	9.7	5	12.9	5	7.3	5.1–10.1	10.0
10 gamma (65 μ) filtered amino acid plus 10 gamma pyrimidine	4	14.1	5	3.7	5	6.7	0.2–12.1	8.2
30 gamma (195 μ) filtered amino acid	5	5.6	5	5.4	4	9.6	0.5–10.7	6.9

amino acid was superior to that in the solution 3 containing the amino acid filtered sterile. In neither however was growth as good as with the thiamin thiazole solution 4. Growth in solution 6 containing 30 gamma of the amino acid per flask was better than with 10 gamma.

At the end of 6 weeks subcultures were made from the best roots in each series into solutions of the same composition and prepared in the same way. Because of the small amount of growth in solution 1 no subcultures could be made and fragments of a fresh root which had grown in a thiamin solution were used. The results in the second passage were in general like those in the first passage.

At the end of 7 weeks fresh solutions of the amino acid were prepared and subcultures of the best root in each series were again made into solutions of the same composition. The results in the third passage were much like those in the first and second. The dry weights of the roots are given in table I and typical roots in each solution at the end of the third passage are shown in figure 1.

In all three passages (table I) the effect of the 10 gamma of thiamin thiazole was superior to that of the amino acid; the 10 gamma of auto-

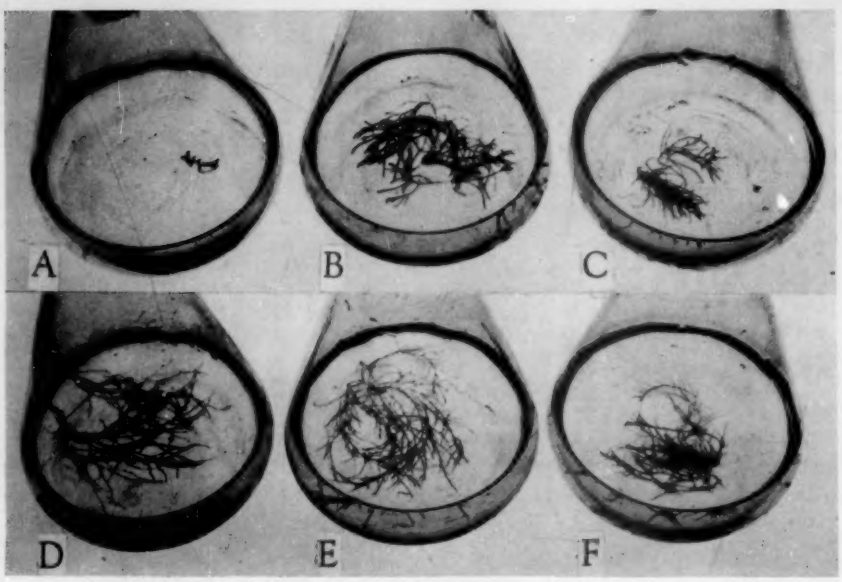


FIG. 1. Growth of tomato roots in a mineral solution containing 2 per cent. cane sugar supplemented with A, nothing; B, 10 gamma thiazolyl alanine autoclaved; C, 10 gamma thiazolyl alanine filtered sterile; D, 10 gamma thiamin thiazole; E, 10 gamma thiazolyl alanine filtered sterile + 10 gamma thiamin pyrimidine; F, 30 gamma thiazolyl alanine filtered sterile. Age, 76 days. 3rd successive passage.

claved amino acid were superior to the same amount of filtered amino acid and the 30 gamma of amino acid were superior to the 10 gamma.

It would appear from these results that the amino acid supplies the needs of the tomato root though not as satisfactorily as the thiamin thiazole. In fact, the habit of growth and general morphology of the roots in the solutions containing the amino acid were much the same as those of roots grown in the presence of the thiamin thiazole. BONNER and BUCHMAN found the amino acid effective with pea roots. On the other hand both Phycomyces and *Staphylococcus aureus* are reported to be unable to use the amino acid appreciably.

What is the explanation for the difference in the response of excised root cultures and of the microorganisms? I believe the difference is apparent only. I have found the amino acid effective with Phycomyces if enough of it is supplied, although amounts of the amino acid of the order of 1000 times those of the vitamin thiazole are required to produce the same effect.[2] This is illustrated by the experiments summarized in table II.

TABLE II

AVERAGE DRY WEIGHTS OF TRIPLICATE CULTURES OF PHYCOMYCES GROWN 6 DAYS AT 25° C. SUPPLEMENTS ADDED PER FLASK TO A SOLUTION OF SUGAR, ASPARAGINE AND MINERAL SALTS ARE GIVEN IN UNITS OF 10^{-9} GRAM-MOLE

ADDITIONS TO SOLUTION OF SUGAR, MINERAL SALTS AND ASPARAGINE	AVERAGE DRY WEIGHT	
	EXPERIMENT I	EXPERIMENT II
	mg.	*mg.*
1 μ thiamin	106.0	84.5
10 μ thiamin	117.0	.....
1 μ thiazole + 1 μ pyrimidine	102.6	93.7
10 μ thiazole + 10 μ pyrimidine	122.0	.....
1 μ filtered amino acid + 1 μ pyrimidine	6.3	.....
10 μ filtered amino acid + 10 μ pyrimidine	10.7	.....
1 μ heated amino acid + 1 μ pyrimidine	7.0	.....
10 μ heated amino acid + 10 μ pyrimidine	9.8	.....
100 μ filtered amino acid + 10 μ pyrimidine	.....	38.8
1000 μ filtered amino acid + 10 μ pyrimidine	.....	105.3
100 μ heated amino acid + 10 μ pyrimidine	.....	38.8
1000 μ heated amino acid + 10 μ pyrimidine	.....	106.0

In these experiments the plus strain of Phycomyces was grown in 125–ml. Erlenmeyer flasks in 25 ml. of a solution containing per liter 50 gm. dextrose, 2.0 gm. asparagine, 1.5 gm. KH_2PO_4, 0.5 gm. $MgSO_4 \cdot 7H_2O$ and the following trace elements 0.01 p.p.m. B, 0.04 p.p.m. Cu, 0.20 p.p.m. Fe, 0.02 p.p.m. Ga, 0.02 p.p.m. Mn, 0.02 p.p.m. Mo, and 0.18 p.p.m. Zn.

[2] Since the preparation of this manuscript BUCHMAN has advised me that BONNER also found the amino acid to have about 1/1000 the activity of the vitamin thiazole for Phycomyces and that KNIGHT found 10^{-3} M concentration to have no effect on Staphylococcus whereas 10^{-6} M concentration of the vitamin thiazole produced noticeable growth.

The cultures were incubated at 25° C. in faint diffuse day light. Various amounts of thiamin, mixtures of the thiamin thiazole and thiamin pyrimidine and of the amino acid and thiamin pyrimidine were added to the basic solutions. Dry weights were determined at the end of 6 days and are given in table II.

With 10^{-9} or 10^{-8} gram-mole of the amino acid growth was entirely submersed and scanty; with 10^{-7} gram-mole aerial mycelium and short sporangiophores were produced; with 10^{-6} gram-mole normal sporangia were formed and growth was as good or better than that produced by 10^{-9} gram-mole of thiamin thiazole. No difference was evident between the effects of the amino acid filtered sterile and that heated in the autoclave. It appears, therefore, that the amino acid is effective with Phycomyces if sufficient is used.

It would seem that the amino acid is much more available to the tomato root than to Phycomyces. A similar difference, however, appears to exist in the relation of these organisms to the vitamin thiazole. The lower limit for a response by Phycomyces to a thiazole pyrimidine mixture is about 10^{-11} gram-mole; a definite and measurable effect on the growth of excised tomato roots of amounts of thiazole as low as 10^{-13} gram-mole has been observed by the writer. This difference in sensitivity to thiazole may be associated with the slower growth and smaller total growth of tomato roots as compared to Phycomyces or it may be the result of some more fundamental difference.

BONNER and BUCHMAN (1) state that the amino acid is transformed *in vivo* and by the pea root into the thiamin thiazole. It is possible, however, that for Phycomyces the amino acid functions as such but is much less effective than the thiamin thiazole or that a transformation into the thiamin thiazole occurs *in vitro*. The latter possibilities appear the more probable. If Phycomyces, for example, is capable of transforming the amino acid into the thiamin thiazole one would rather anticipate a greater transformation than the growth reported here indicates. The observation that the autoclaved amino acid was more effective with tomato roots than that which was filtered sterile may have resulted from some adsorption in filtration or from a partial change of the amino acid into the thiamin thiazole *in vitro*. Failure to observe a similar effect with Phycomyces may have been because of the greater thiazole requirement of that organism. In any event, we can not yet accept the assumption that the amino acid is a normal precursor of the thiamin thiazole *in vivo*.

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SPECIFICITY OF NICOTINIC ACID AS A GROWTH FACTOR FOR ISOLATED PEA ROOTS[1]

JAMES BONNER
(WITH ONE FIGURE)

It is known that nicotinic acid is essential to the growth of numerous species of isolated roots (ADDICOTT and BONNER, **1**, BONNER and DEVIRIAN, **4**). The present paper is concerned with the chemical specificity of nicotinic acid as a root growth factor.

Methods

The pea roots (variety Perfection) which were used exclusively in the present experiments were cultured according to the methods outlined in earlier papers (BONNER and ADDICOTT, **3**; BONNER and DEVIRIAN, **4**). Four-mm. tips were cut from the roots of aseptic pea seedlings and transferred to nutrient medium containing per liter of redistilled water 236 mg. $Ca(NO_3)_2 \cdot 4H_2O$; 36 mg. $MgSO_4 \cdot 7H_2O$; 81 mg. KNO_3; 65 mg. KCl; 20 mg. KH_2PO_4; 1.5 mg. ferric tartrate; and 40 gm. of sucrose. After the tips had remained in the medium for one week at 25° C., and had grown into roots several cm. long, they were subcultured by tranferring 1-cm. tips to fresh nutrient solution. This process of subculture was repeated weekly. Vitamin B_1, which is essential to the continued growth of isolated pea roots, was added at the rate of 0.1 mg. per liter to all of the nutrient solution for transfers later than the first. Nicotinic acid, or related substance to be tested for ability to replace nicotinic acid, was added at the rate of 0.5 mg. per liter, since this concentration has been found to be approximately optimal for pea roots (ADDICOTT and DEVIRIAN, **2**).

A rigid selection for uniformity in root growth rate was carried out at the end of the first week of culture in order to assure the greatest possible uniformity in the roots actually used in each experiment. In a single experiment 400–500 roots were cultured and 20–50 roots were used for testing the activity of each substance reported below. In each experiment the roots were maintained through 5 or more weekly transfers.

Experimentation

The ability of decreasing concentrations of nicotinic acid to support the continued growth of isolated pea roots is shown in figure 1. Although 0.5 γ nicotinic acid per liter of nutrient solution supports modest growth, a ten times higher concentration is needed to support growth at a significant level. Still ten times higher concentration (50 γ per liter) supports growth

[1] Work carried out with the assistance of the Works Progress Administration, Official Project Number 65–1–07–98, Work Project Number 11534.

at a maximum level, beyond which further increases result in no further increases in growth rate. The standard concentration, 0.5 mg., of nicotinic acid used in these experiments is therefore at least 100 times as high as is needed for a detectable promotive influence on the growth of isolated pea roots. In the experiments reported later analogs of nicotinic acid were also used in a concentration of 0.5 mg. per liter. An analog inactive at this concentration may then be concluded to possess less than 1 per cent. of the activity of nicotinic acid in supporting the growth of isolated pea roots.

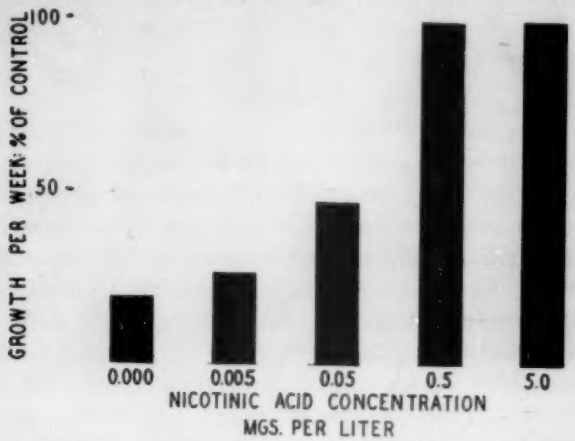

FIG. 1. Growth of isolated pea roots in medium containing adequate amounts of vitamin B_1 and varying amounts of nicotinic acid. The growth rates given are those for the fifth week of roots which had already been maintained through five weekly transfers in a medium of the composition shown.

Table I gives as an example one experiment in which methyl nicotinate and β-picoline respectively were substituted for nicotinic acid in the basic medium. In the absence of nicotinic acid growth in length decreases in each successive transfer. In medium containing nicotinic acid on the other hand, the growth rate is maintained at essentially a constant level. After 5 weekly transfers the roots which received nicotinic acid grew approximately 5 times as much per week as roots which did not receive nicotinic acid. Table I shows that the methyl ester of nicotinic acid also supports the growth of isolated pea roots and it may be concluded that methyl nicotinate is capable of replacing nicotinic acid as a growth factor for the isolated pea root. β-picoline, on the other hand, is clearly incapable of replacing nicotinic acid since roots supplied with β-picoline grew no better than the control roots which received vitamin B_1 alone.

All of the substances discussed below were tested, in experiments similar to that exemplified in table I, for ability to support the growth of pea roots through at least 5 weekly transfers. Each substance was either clearly inac-

TABLE I

ACTIVITIES OF 2 SUBSTANCES RELATED TO NICOTINIC ACID IN SUPPORTING THE CONTINUED GROWTH OF ISOLATED PEA ROOTS. 30–40 ROOTS IN EACH SERIES. ROOTS CULTIVATED FOR A PRELIMINARY WEEK IN MEDIUM CONTAINING VITAMIN B_1 AND NICOTINIC ACID

SUPPLEMENTS TO MEDIUM	GROWTH PER WEEK DURING:				
	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII
	mm.	mm.	mm.	mm.	mm.
Vitamin B_1, 0.1 mg./l.	63	42	35	22	16
" + nicotinic acid, 0.5 mg./l.	79	78	74	86	80
" + methyl nicotinate, 0.5 mg./l.	85	78	81	96	78
" + β-picoline, 0.5 mg./l.	58	41	31	22	16

tive and supported growth at no higher level than nicotinic acid-free medium or it was completely active and supported growth essentially as well as nicotinic acid itself. Table II gives the activities in supporting isolated pea root growth of 23 substances related to nicotinic acid. Nicotinamide, esters of nicotinic acid, and nicotinuric acid were the only compounds among the substances tested which were active in supporting the growth of isolated pea roots. These active substances are all ones which yield nicotinic acid on simple hydrolysis. It must be concluded therefore that of the substances tested, only those which readily yield nicotinic acid by hydrolysis *in vivo* are capable of replacing the latter substance in the nutrition of isolated pea roots.

TABLE II

ACTIVITIES OF SUBSTANCES RELATED TO NICOTINIC ACID IN SUPPORTING THE GROWTH OF ISOLATED PEA ROOTS. + = ACTIVE. 0 = INACTIVE. DATA OBTAINED BY EXPERIMENTS OF TYPE SHOWN IN TABLE I

1. Nicotinic acid	+	10. Picolinic acid[1]	0
2. Nicotinamide	+	11. Quinolinic acid[1]	0
3. Coramine (N-diethyl amide of 1)[1]	0	12. Dinicotinic acid[1]	0
4. Methiodide of 2[1]	0	13. Nicotinuric acid[1]	+
5. Methyl nicotinate[1]	+	14. Ethyl nicotinoylacetate[1]	0
6. Ethyl nicotinate[1]	+	15. Arecoline[1]	0
7. Propyl nicotinate[1]	+	16. β amino pyridine[1]	0
8. Butyl nicotinate[1]	+	17. Nicotino-3-nitrile[1]	0
9. Isonicotinic acid[1]	0	18. β-picoline[1]	0
		19. Thiazole-5-carboxylic acid[2]	0
		20. Amide of 19[2]	0
		21. Benzoic acid	0
		22. Pyrazine, 3, carboxylic acid[2]	0
		23. Pyrazine, 2–3, dicarboxylic acid[2]	0

[1] Obtained through the courtesy of Prof. FELIX SAUNDERS and Dr. ALBERT DORFMAN, University of Chicago.

[2] Obtained through the courtesy of Dr. FRANZ C. SCHMOLKES, Research Division, Wallace & Tiernan Products, Inc., Belleville, New Jersey.

Inspection of table II shows that the steric requirements for nicotinic acid activity are strict. Thus isonicotinic acid and picolinic acid are completely inactive, although they differ from nicotinic acid only in the position of the carboxyl group relative to the heterocyclic nitrogen atom. Substitution of a second carboxyl group as in dinicotinic acid and quinolinic acid also renders the molecule inactive. It is noteworthy that the N-diethyl substituted amide of nicotinic acid, coramine, is inactive, indicating that this substance is not hydrolyzed *in vivo* by the pea root.

The carboxylic acids of certain cyclic substances other than pyridine are unable to replace nicotinic acid in the nutrition of pea roots. Thus benzoic acid is inactive. Thiazole-5-carboxylic acid and its amide are both inactive even though in this substance, as in nicotinic acid, the carboxyl group is β to the cyclic N atom. Pyrazine-3-carboxylic acid is likewise inactive.

Vitamin B_6, which like nicotinic acid is a pyridine derivative, is completely incapable of replacing the latter in the nutrition of isolated pea roots. Similar results have been obtained with isolated tomato roots (**10**).

Discussion

In relation to the general question of which reactions are capable and which incapable of execution *in vivo*, it is of interest that the pea root can neither hydrolyze nicotino-3-nitrile nor oxidize the methyl group of β-picoline to form nicotinic acid.

The specificity of nicotinic acid in the nutrition of pea roots is in substantial agreement with the specificity of this substance in the nutrition of *Staphylococcus aureus* (**7, 8, 9**), dysentery bacillus (**5, 6**) and the dog (**11**), with the particular exception that β-picoline is claimed to possess curative activity for black tongue.

The present data do not permit of a decision as to whether it is nicotinic acid *per se* which is required as a growth factor by the isolated pea root, or whether it may not be nicotinamide which, as a constituent of the codehydrogenases (**12**), is the true active substance. On the assumption that nicotinamide is the substance actually utilized by the pea root, it must be concluded that the root is capable of ready amide formation *in vivo*.

Summary

The ability of 23 substances chemically related to nicotinic acid to replace the latter substance as a growth factor for isolated pea roots has been determined. Among those tested, only substances which yield nicotinic acid by simple hydrolysis were found to be active.

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BRIEF PAPERS

TRANSMISSION SPECTROGRAMS OF LEAF EXTRACTS

FREDERICK F. FERGUSON, WILL S. DELOACH, AND LEWIS W. WEBB, JR.

(WITH TWO FIGURES)

It is felt that biologists in general, and plant physiologists in particular, might be interested in the transmission spectrogram of a denatured alcoholic solution of leaf extracts, which solution is commonly used in the laboratory for demonstration purposes. The following curves were obtained by the

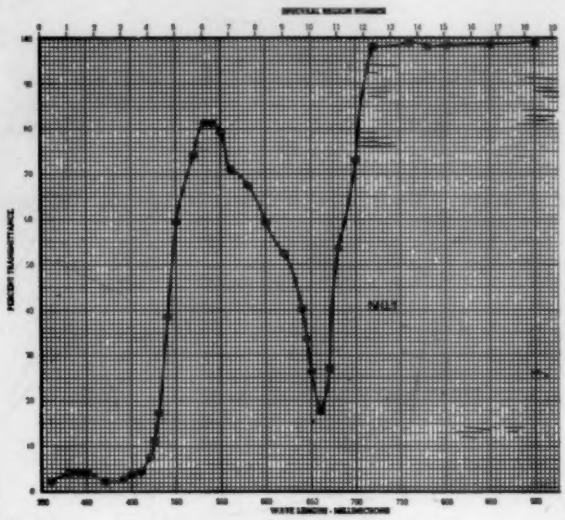

FIG. 1. Transmission spectrogram of *Poa pratensis* leaf extract using 30 mμ bands.

use of the Coleman Regional Spectrophotometer.[1] The points recorded in figure 1 represent the average transmission of bands 30 mμ in width.

The solution used was prepared in the following manner: denatured ethyl alcohol (100 ml.) was added to selected fresh green leaves of *Poa pratensis* (2 gm.) and the resulting mixture was heated for 15 minutes on a water bath under a reflux condenser. The dark green alcoholic solution was decanted and diluted by the addition of 15 parts of denatured alcohol to 5 parts of the solution. This dilution was made in order to get appreciable transmission in the region from *ca.* 360 mμ to *ca.* 450 mμ.

[1] This instrument was procured for this study through the kindly offices of the Phipps and Bird Co., Richmond, Virginia.

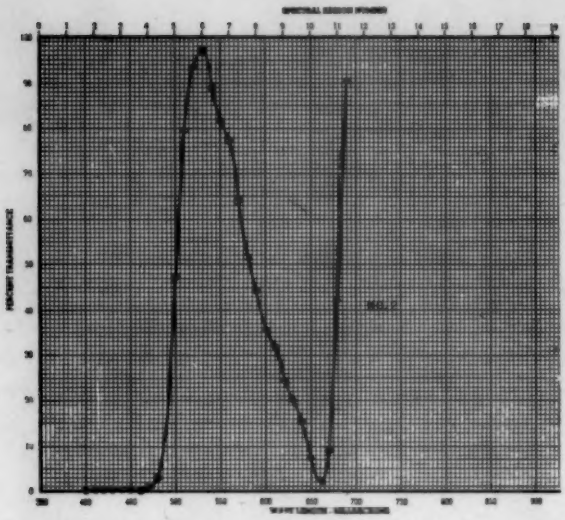

FIG. 2. Transmission spectrogram of dilute leaf extract of *Poa pratensis* using 10 mμ bands.

Figure 2 was secured by the use of bands of approximately 10 mμ in width. In this case a more dilute solution was used in order to allow the passage of more light.

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IMPROVEMENTS IN THE SIMPLIFIED METHOD FOR OSMOTIC MEASUREMENTS

CHARLES J. LYON

The extension of URSPRUNG's simplified method (2) to the provision of data for determining osmotic quantities other than net osmotic pressure, was suggested in 1936 (1). At that time we described the immersion of cylinders of tissue, each several centimeters long, in test tubes of sugar solutions. Each cylindrical stick was measured with rule and hand lens and the length used as an index of the volume of the cylinder. Subsequent use of the method for research and classroom purposes has led to refinements in procedure.

In the place of long cylinders, it is better to cut sticks approximately square in cross section and somewhat less than 1 cm. in length. These are easily cut to the same length by use of a double-bladed knife, made by bolting two razor blades to a block of suitable width. A slice of desired thickness, preferably one-tenth the cut of the double knife, is first taken from the tuber, fruit, or other organ by a sharp, broad-bladed knife. The double knife is used to make parallel cuts through the slice; from the tissue between them, a razor blade held vertically is used to take a series of square sticks of equal length.

In place of stoppered test tubes from which the tissue must be removed for measurement, the short, square sticks should be immersed in low preparation dishes. They can then be measured accurately without danger of drying or distortion by setting each dish in turn in the field of a 10× binocular microscope provided with a micrometer scale in one ocular. It is convenient to use 10 or 12 ml. of water, or solution, in each dish. If plant material is plentiful, several sticks of it may be placed in each dish to lower the sampling error.

It is important to hold a few control sticks of the tissue in similar dishes of paraffin oil unless the oil kills the cells rapidly. For undetermined reasons, these control sticks may shrink or expand between the start and the end of the immersion period, which need not be over 4 hours for the short sticks recommended. The final measurement of the control is the logical figure to use for the original length of stick unless the cells die and the walls contract.

If the distilled water kills the cells in the test of volume at the saturation point, no significant osmotic error is introduced by the use of good tap water. Tissues sensitive to distilled water will usually live in tap water and the volume at the saturation point is essential to the computations.

For exact work with the method, the series of solutions must be closely graded. This usually means a preliminary test to determine the approxi-

mate location of the two critical points—incipient plasmolysis and the "normal" of the tissue as it came from the plant. The point of incipient plasmolysis is always the more difficult to locate and very important for all estimates of absolute osmotic values.

It is possible to improve the accuracy of this method for determining osmotic quantities by using calculated volumes for the sticks of tissues at the three critical points. In the original description of the procedure, the length was used as an index of the volume. It is impractical to measure more than the length of each stick but if the side of the square end is made approximately one-tenth the length, it is easy to calculate a satisfactory volume by the use of a table of cubes.

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ISOLATION OF THE ALKALOIDS, BERBERINE AND BERBAMINE, FROM *MAHONIA SWASEYI*

GLENN A. GREATHOUSE AND NEIL E. RIGLER

In a previous communication, the senior author (**1**) recorded the isolation of berberine, $C_{20}H_{17}O_4N \cdot 2\frac{1}{2} H_2O$ from *Mahonia swaseyi*, Fedde (*Berberis swaseyi* Buckl.). Further studies have revealed the presence of the alkaloid berbamine in the root tissue of this species. *Mahonia swaseyi* is of limited habitat and has not been hitherto chemically examined. The resistance of this plant to *Phymatotrichum omnivorum* (Shear) Duggar and its use as an ornamental shrub in root rot areas of Texas were factors in the initiation of these chemical examinations.

The procedure for the separation of berberine has been described (**1**). Following the separation of berberine, berbamine remained in the mother liquors and was removed by addition of sodium hydroxide solution, and extraction by ether. The residue left on evaporation of the solvent was dissolved in acetic acid. From this solution, berbamine nitrate was precipitated with sodium nitrate. The alkaloid was regenerated, converted into the hydrochloride, and recrystallized from water. No other alkaloids were detected in these studies. Berberine was found (**1**) to be present in a concentration varying from 2.15 to 2.48 per cent. on the dry weight basis. Berbamine, on the other hand, was present in concentrations in the root tissue to the extent of 0.02 to 0.05 per cent.

The color reactions with H_2SO_4, and HNO_3, and the melting point of 200 to 202° C. (corrected) of the free base recrystallized from petroleum ether are those recorded in the chemical literature for berbamine.

Calculated: for $C_{18}H_{19}O_3N$,—N, 4.71%; H, 6.45%; C, 72.68%. Found:[1] N, 4.60%; H, 6.51%; C, 72.82%.

The localization of berberine and its fungicidal effectiveness on the growth of *P. omnivorum* has been reported (**1, 2**). The fungicidal power of berbamine to this fungus has been also recorded (**2**).

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CRITICISM OF A RECENT PAPER ON THE PECTIC CONTENT OF PLANT MATERIALS

Z. I. KERTESZ

A paper entitled *Pectic content of plant materials* appeared in PLANT PHYSIOLOGY a few months ago (**2**). ELWELL and DEHN analyzed various plant tissues and vegetables to determine the proportion of pectic constituents present. The material was first extracted with water, then twice with dilute sulphuric acid. The extracts were used for the determination of pectic materials as calcium pectate (**1**) and by alcohol precipitation. The results obtained on the three extracts were combined to give the total pectin content of the analyzed material. The following criticisms are offered in connection with the techniques employed by ELWELL and DEHN.

1. An alcohol precipitate from an extract from a plant tissue, or even from a fruit, may include a great many other compounds besides the pectins. Starch, dextrins, hemicelluloses, proteins, and many other plant constituents are extracted by hot water and precipitated by the addition of alcohol. Thus while the precipitates undoubtedly *included* pectins, the pectin may have constituted only a fraction of the total alcohol precipitate, designated in the article as pectin.

2. While the unspecific precipitation by alcohol includes many materials in addition to pectin, the determination of the pectins as calcium pectate, if properly carried out, is specific for the pectic materials. Consequently the percentage of alcohol precipitate obtained from crude plant extracts is always higher than the percentage of calcium pectate determined in the same extract. From the 15 materials analyzed by ELWELL and DEHN by both methods the percentage of "pectin as calcium pectate" is in nine cases higher than the percentage of total alcohol precipitate.

This discrepancy between the two methods is most apparent from the results given in table I for the fresh and stored cranberry (berry). The results, expressed by the authors on the dry matter basis, are as follows:

	PECTIN AS CALCIUM PECTATE	PECTIN AS ALCOHOL PRECIPITATE
	%	%
Cranberry, fresh	50.6	2.74
Cranberry, stored	16.53	9.77

An observation of the results makes further discussion of this point superfluous.

3. The percentage of "pectin as alcohol precipitate" (it should be "alcohol precipitate as pectin") as reported by ELWELL and DEHN is in many

cases so high that it will be obvious to all concerned with the analysis of plant materials that the figures are either incorrect or do not represent "pectin." For instance, the solids of asparagus are claimed to contain 28.54 per cent.; dandelion greens 31.42 per cent.; lettuce 24.99 per cent.; rhubarb 90.9 per cent. (!) and Swiss chard 70.80 per cent. pectin. Again no discussion of these percentages is needed to demonstrate the obvious impossibility of these results.

It appears from the foregoing that ELWELL and DEHN either overlooked an important detail in the chemical methods used or that some mistake was made in calculating the results. In either case it would be most unfortunate if the alleged pectin contents of various plant materials, as reported by these authors, should be accepted and incorporated in the already confused and contradictory literature of pectic materials.

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NOTES

Annual Election.—The results of the seventeenth annual election of the American Society of Plant Physiologists have just been announced by the Secretary, Dr. W. E. Loomis, Iowa State College, Ames, Iowa. His report names Dr. Frank P. Cullinan, U. S. Horticultural Station, Beltsville, Maryland, as president for 1940–41; and Dr. Bernard S. Meyer, Ohio State University, as vice-president.

Dr. John W. Shive, who has just retired from the presidency, was elected to the Executive Committee for a term of three years, and Dr. H. R. Kraybill, Purdue University, was reelected to the editorial board for a three-year period.

These newly elected officers hold very responsible positions, which will present many opportunities for service. It is a pleasure to extend congratulations and to express the hope that the duties may not seem too arduous. The cooperation of the entire membership is assured.

New England Section.—The 1940 meeting of the New England Section of the American Society of Plant Physiologists was held at Dartmouth College, Hanover, New Hampshire, on May 10–11. The usefulness and value of the sectional meeting is indicated by the fact that 20 institutions were represented in the gathering, and more than 100 investigators were able to exchange ideas and discuss their mutual problems. The voluntary submission of papers brought out 28 contributions, which were presented in four sessions. Dr. Charles J. Lyon of Dartmouth was in charge of the arrangements, and also performed the pleasant duty of introducing President John W. Shive of the A.S.P.P., who brought greetings from the national organization. The evening program was distinguished by an illustrated lecture by Jack Durrance (Dartmouth, '39) on his famous trip through India and the climbing of "K-2" in the Himalayas.

Of special value in all such gatherings are the friendly and eager discussions that take place informally outside the regular sessions. It is common experience to gain more from these informal exchanges than from the formal programs.

The officers for the coming year were elected as follows. Chairman, Dr. Thomas G. Phillips, University of New Hampshire; vice chairman, Dr. Dorothy Day, Smith College; and secretary treasurer, Dr. Linus H. Jones, Massachusetts State College.

Charles Frederick Hottes.—At the sixteenth annual meeting of the American Society of Plant Physiologists held at Columbus, Ohio, it was voted by the Society to dedicate one of the numbers of Plant Physiology

during 1940 to Dr. CHARLES FREDERICK HOTTES, who celebrates the seventieth anniversary of his birth on July 8, 1940. The July number is therefore dedicated to him in honor of this occasion. Dr. HOTTES, son of FREDERICK and BARBARA (DATHAN) HOTTES, was born at Mascoutah, Illinois, a small town in St. Clair County. Following his education in the public schools, he entered the University of Illinois, from which institution he was graduated with a B.S. degree in 1894. He continued work in the graduate school at Illinois with the aid of a fellowship, and was awarded the M.S. degree in 1895. His excellent work led to his appointment to an assistantship in the Department of Botany of his alma mater, a position he held for three years. In August, 1895, he was married to Miss FLORA GUTH, also of Mascoutah. Their only child, a daughter, FLORA EMILY, lives at Kenosha, Wisconsin, where she is engaged in library work. During these early years, those who aspired to the doctorate in botany were accustomed to go to European universities. Dr. HOTTES chose to go to Bonn, where he received the M.A. and Ph.D. in 1901.

Returning to the University of Illinois, he was appointed to an instructorship for one year (1901–02) and became assistant professor in 1902, professor in 1913, consulting plant physiologist to the Department of Agronomy in 1923, and finally head of the Department of Botany in 1928, a position he held for 10 years, until his retirement. His connection with the University of Illinois as student and professor extended through a period of sightly less than a half century.

Dr. HOTTES is noted for his studies of cellular physiology and experimental cytology, and for his ingenuity in developing apparatus for the control of cellular environment, and for control of general environmental conditions. From his studies he has accumulated a large amount of valuable information, much of which unfortunately is still unpublished. Many monographs and books would be required to record the detailed results of his work; but he has refrained from publication, a serious loss to all of us who need to know what he knows about cellular responses.

One quality Dr. HOTTES possesses which makes him a rare teacher—his contagious enthusiasm for his subject. He held his classes spellbound by his masterly presentation of things not to be found in books. He could illustrate his lectures, and illuminate the discussions, with deep personal experiences and unique observations which gave distinction to his teaching.

Many students were attracted to the field of botany and to plant physiology because of his abiding enthusiasm for both plant physiology and students. He also gave unstintingly of time and energy to community interests and problems, and was always available to those who sought his services in a public enterprise.

He was a member of the American Society of Plant Physiologists very shortly after it was organized, and published a paper, *Studies in Experi-*

mental Cytology, in PLANT PHYSIOLOGY in 1929. At the Atlantic City meeting in 1932, he was honored by the Society as the eighth recipient of the CHARLES REID BARNES life membership award in recognition of his life-long services to plant physiology, an honor richly merited.

The portraits reproduced in this number of PLANT PHYSIOLOGY were obtained through the kind cooperation of Professor JOHN T. BUCHHOLZ, Dr. HOTTES's successor as head of the Department of Botany at the University of Illinois. The portrait in plate IV is reproduced from a photograph of a painting which was presented to the University of Illinois by his former students at the time of his retirement. The original painting hangs in the reading room of the Natural History Building. This photograph scarcely does the painting justice. It represents the serious professor.

The photograph from which plate V was made dates from about 1935, and shows him as genial companion and friendly counsellor of students at the zenith of his career. It is a pleasure to be able to share these fine portraits with our readers.

On behalf of all members of the Society, and all of Dr. HOTTES's legion of friends, we extend to him and to his family most cordial greetings and good wishes on the happy occasion of his seventieth birthday anniversary, for long continued health and prosperity.

Rodney Howard True.—Again death has invaded the ranks of the life members of the American Society of Plant Physiologists. Everyone who knew him will feel keenly the loss of Dr. RODNEY HOWARD TRUE, who died at Philadelphia on April 8, 1940, in his 74th year. Dr. TRUE was an early member of the Society, and was the sixth recipient of the CHARLES REID BARNES award. This honor was bestowed upon him at the Cleveland meeting in 1930. A brief biography of TRUE was published in PLANT PHYSIOLOGY, volume 6, 197–198, 1931, following the award, and again in volume 12, 223, 1937, in honor of the seventieth anniversary of his birth, which occurred on October 14, 1936. A fine portrait of Dr. TRUE adorns the January, 1937, number of our journal. It is not necessary to add many details of his life at this time. He was deeply interested in the broader aspects of science and the history of science; and as long as he was physically able, he was active in the affairs of the American Association for the Advancement of Science. He is remembered especially for his work as chairman of the Committee of One Hundred.

The last public service which he rendered to the Society was in connection with the 15th award of the BARNES life membership. He was chairman of the award committee, and those who were present at the annual dinner at Richmond will recall the evident satisfaction with which he announced

that the life membership had been awarded to Dr. LUDWIG JOST of Heidelberg.

Dr. TRUE had suffered from heart ailments for a number of years, and his activities had been hampered by them; but he did not retire as emeritus professor of botany at the University of Pennsylvania until 1937, when he was in his 71st year. It was the increasing severity of the heart complications which finally caused his death. To his wife and other members of his immediate family we express our deep sympathy.

Annual Review of Biochemistry.—Volume IX of this valuable annual has been received, and again we commend it to our readers as a volume of extraordinary value. Some of the reviews cover the same regions of biochemistry as were covered in 1939, as *Biological Oxidations and Reductions,* and *Proteolytic Enzymes.* A number of them cover fields that were not reviewed last year. The new reviews, are, however, presented by different authors. Last year DIXON covered the field of oxidations and reductions; this year the advances are summarized by K. G. STERN. By changing authors, no one individual is overworked, and the interpretations have no tendency to become stereotyped.

The 1940 volume, 744 pages, contains 26 reviews, well distributed, and of about equal interest to plant and animal investigators. Of particular interest to plant physiologists are such reviews as *Plant Pigments,* by G. MACKINNEY; *Aspects of Inorganic Metabolism in Plants,* by C. B. LIPMAN; *Organic Acids of Plants,* by H. B. VICKERY and G. W. PUCHER; *Soil Microbiology,* by S. A. WAKSMAN; and *Biochemistry of the Lower Fungi,* by H. RAISTRICK. Most of the other reviews contain material of most general physiological applicability. They have been carefully prepared, and are worthy of careful reading by anyone who desires to keep up with the march of progress in biochemistry.

We recommend it most highly for public, institutional, and personal libraries. The bibliographies are valuable in themselves, and the work is a challenge to anybody's $5.00 bill. No greater value is to be obtained anywhere. It is a standard work, worthy of the support of all physiologists. Orders will be filled by Annual Reviews, Inc., Stanford University, California.

Sulphur Dioxide.—A comprehensive investigation of sulphur dioxide injury to vegetation has been in progress under the auspices of the National Research Council of Canada, for about a decade. The work was undertaken as a fact-finding study of injuries claimed to have been caused to vegetation in the area surrounding the great industrial plants of the Consolidated Mining and Smelting Co. at Trail, British Columbia.

The report of the investigations has been published with the title *Effect of Sulphur Dioxide on Vegetation.* The first half of the report concerns the field studies on the quantity of SO_2 found in the atmosphere near this industrial area; the symptoms of injury on forest and crop plants; the sulphur content of the vegetation in relation to the sulphur dioxide content of the air; hydrogen ion concentration, base exchange capacity, and sulphur content of the regional soils; hydrogen ion concentration and sulphate content of water supplies; and the effect of SO_2 on the diameter increment of conifers. This section of the work is profusely illustrated from photographs showing the characteristic injuries to leaves when the sulphur dioxide exceeds the tolerance of the vegetation. There are 24 excellent plates, two in colors which show the injuries found on needles of the yellow pine and Douglas fir. These conifers are among the more sensitive species.

The second half of the report deals with fumigation experiments. The plots and apparatus used in the experimental investigations are described, and the succeeding chapters discuss fumigation experiments on conifers in their natural habitat; on transplanted conifers; the effects of environmental factors on the susceptibility of barley and alfalfa to sulphur dioxide; stomatal behavior of fumigated alfalfa; experiments on the yield of barley and alfalfa; the effect of fumigation on some chemical constituents of barley, wheat, and alfalfa; and CO_2 assimilation and respiration of alfalfa under the influence of SO_2. The final chapter is a summary, chapter by chapter, of the entire report.

This monograph is a valuable contribution to the subject of SO_2 injuries to vegetation. It contains scientific and practical information that will be extremely useful to investigators and to industrial management. It is bound in handsome blue cloth, and because of the large number of text figures, plates, and the colored plates, it is an expensive publication. Nevertheless, it deserves a wide distribution among plant physiologists, and among our technical libraries. The price quoted is $15.00 per copy; it is obtainable through the National Research Council of Canada at Ottawa.

Methods of Enzyme Research.—The first section of an important compilation of research methods applicable to enzyme chemistry has been received from the press of Georg Thieme, Leipzig. It is appearing under the title *Die Methoden der Fermentforschung.* This first "Lieferung" occupies 172 pages, 8×11. The general introduction, 8 pages, defines terms and presents a short historical survey of the field.

The first "Hauptteil" is a general section, which begins with a consideration of the substrates; and first of all, with the preparation, characteristics, and the investigation of important substrates, intermediates, and end products. The substrates of the ester-splitting enzymes are considered at

the outset, including such as the substrates of lipases; naturally occurring glycerides; acetylcholine; gallotannin and other substrates of tannase; chlorophyll; simple esters of phosphoric acid; inosite phosphoric acid; phosphatides and their hydrolytic products; and simple esters of sulphuric acid. Each of these topics is presented by a collaborating author. The section on chlorophyll is by HANS FISCHER, Munich; and the one on glycerides by KARL HUGO BAUER, Leipzig, etc. The main editors of this elaborate work are Dr. EUGEN BAMANN, Tübingen, and Dr. KARL MYRBÄK, Stockholm.

The last 57 pages of this "Lieferung" are occupied by four papers on biologically important carbohydrates and glycosides. The sugars are discussed by E. L. HIRST and S. PEAT of England; preparation of natural heterosides, by J. RABATÉ, Paris; synthetic glycosides, by HORST ELSNER, Berlin; and thioglycosides, by FRITZ WREDE, Berlin.

It is not known at the present time just what the cost of this valuable work may be, or how rapidly succeeding parts may become available. This first section, 172 pages, is listed at RM 18, and a general price of RM 1.6 for each 16-page folder has been set. There will be about 220 of these, which indicates well over 3000 pages in the completed work. About 6000 references in the text. Purchasers are reminded that those who order the first section are considered to be obligated to the publisher to accept and pay for the entire series of "Lieferungen." Separate sections are not purchasable.

Attention is also called to the price reduction of 25 per cent. for foreign orders, when the payments are made in accordance with stipulated conditions (Devisen oder Freimarke).

The work is most highly recommended to all investigators and to all establishments concerned in enzyme research of any kind. Every institutional library which caters to investigational needs should subscribe for it as an indispensable item. Orders should be placed with the publisher, Georg Thieme, Publisher, Leipzig.

Annual Review of Physiology.—The second volume of the *Annual Review of Physiology* contains twenty reviews, each one dealing with the recent progress of research in some special phase of animal or human physiology. A few representative titles are as follows: Developmental physiology; respiration; the digestive system; the lymphatic system; energy metabolism; bioelectric potentials; endocrine glands; mammalian reproductive organs; exercise; and physiological psychology.

These reviews have been carefully prepared, and give an adequate account of the recent additions to our knowledge in the fields presented. They are valuable because of the time saved for investigators, teachers, and students who must obtain in a short time a clear picture of the front-line situa-

tions. This annual has taken its place along with the *Annual Review of Biochemistry* as an invaluable aid in the progress of physiology. The editors and authors deserve much credit for this excellent contribution to science. The volume contains 501 pages, and is sold at $5.00 per copy by Annual Reviews, Inc., Stanford University, California.

Trees and Shrubs.—Horticulturists and plant physiologists must often use cultivated varieties of trees and shrubs in their investigations. Taxonomic problems frequently confront the worker who desires to state definitely what variety he used for his studies. The second edition of Dr. ALFRED REHDER'S *Manual of Cultivated Trees and Shrubs*, which was released on June 4, 1940, by the Macmillan Co., will be a welcome aid in solving these taxonomic difficulties. It offers a synopsis of the orders and families covered, and an analytical key to the families and aberrant genera. The main body of the manual, of course, provides the names and descriptive characterizations of a very large number of cultivated woody plants. This edition includes many new introductions and hybrids, and also incorporates changes made desirable by changes in the rules of nomenclature adopted by the Botanical Congresses at Cambridge and Amsterdam.

The revision of such a work requires a vast amount of labor, and Dr. REHDER has rendered a great service to his fellow botanists who owe him a debt of gratitude for this excellent source of information. The price quoted for the new edition is $10.50 per copy. Orders may be sent to the Macmillan Co., New York.